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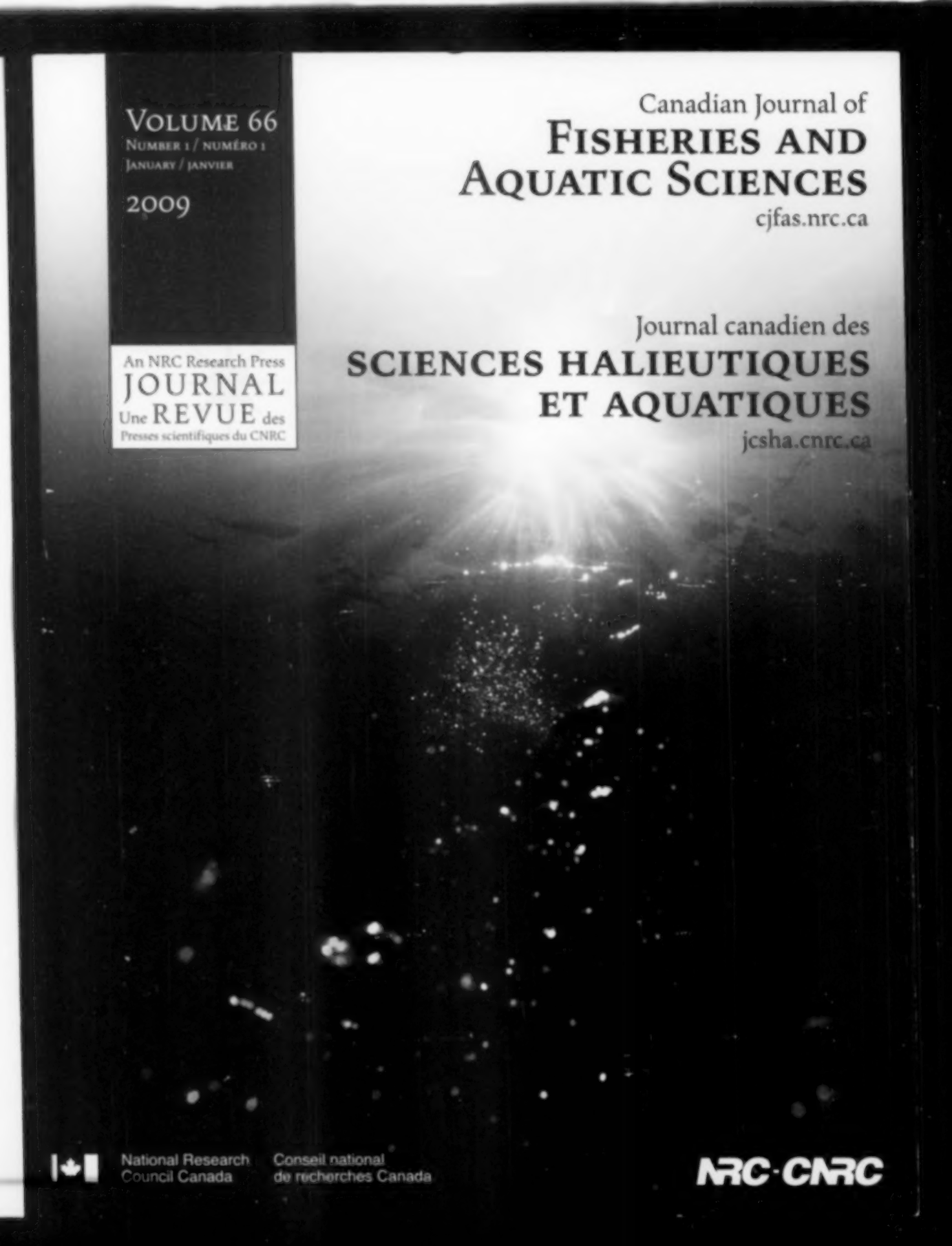

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Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis

M. Robbins Church, Joseph L. Ebersole, Kirk M. Rensmeyer, Ryan B. Couture, Frederic T. Barrows, and David L.G. Noakes

Abstract: Stable isotope analysis of diet switching by fishes often is hampered by slow turnover rates of the tissues analyzed (usually muscle or fins). We examined epidermal mucus as a potentially faster turnover "tissue" that might provide a more rapid assessment of diet switching. In a controlled hatchery experiment, we switched the diet of juvenile steelhead (sea-run rainbow trout, *Oncorhynchus mykiss*) from a plant-based feed with low $\delta^{13}C$ and $\delta^{15}N$ to a fish-meal-based diet with higher delta values. We found mucus to provide a significantly more rapid response to diet switching (half-life = 36 days for $\delta^{15}N$, 30 days for $\delta^{13}C$) than muscle tissue (half-life = 94 days for $\delta^{15}N$, 136 days for $\delta^{13}C$), even for growing juvenile fish. Mucus may provide a rapid turnover "tissue" for analysis of diet (or habitat) switching by fish. It has the additional advantage that it may be sampled nonlethally in some fishes, thereby avoiding problems in studying threatened or endangered species. This is the first report of the use of fish mucus in stable isotope analyses of fish tissues.

Résumé : Les analyses de changements de régime alimentaire chez les poissons au moyen des isotopes stables sont souvent entravées par les faibles taux de remplacement des tissus analysés (généralement les muscles ou les branchies). Nous évaluons l'utilisation du mucus épidermique comme « tissu » à taux de renouvellement potentiellement plus élevé qui pourrait fournir une appréciation plus hâtive des changements de régime. Dans une expérience en conditions contrôlées de pisciculture, nous avons modifié l'alimentation de jeunes truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) d'un régime à base de plantes à $\delta^{13}C$ et $\delta^{15}N$ bas, à un régime à base de farine de poisson avec des valeurs plus élevées des deltas. Le mucus réagit significativement plus rapidement au changement de régime (demi-vie = 36 jours pour $\delta^{15}N$ et 30 jours pour $\delta^{13}C$) que le muscle (demi-vie = 94 jours pour $\delta^{15}N$ et 136 jours pour $\delta^{13}C$), même chez les jeunes poissons en croissance. Le mucus peut donc constituer un « tissu » à taux de renouvellement rapide pour l'analyse des changements de régime (ou d'habitat) chez les poissons. Il présente, de plus, l'avantage de pouvoir être échantillonné chez certains poissons sans devoir sacrifier l'animal, ce qui évite quelques-uns des problèmes associés à l'étude des espèces menacées ou en voie de disparition. C'est la première fois que le mucus de poisson est utilisé pour l'analyse des isotopes stables dans les tissus de poisson.

[Traduit par la Rédaction]

Introduction

The analysis of stable isotope ratios in fish can be a powerful tool for studying fish ecology, including, for example, movement of fish from one habitat (and diet) to another (e.g., Maruyama et al. 2001) or diet switching by a resident species to make use of migratory prey (e.g., MacAvoy et al. 2001). Studies of diet switching by vertebrates (Dalerum and Angerbjörn 2005) and fishes in particular (Herzka 2005) often are hampered by rates of growth or tissue turnover that are relatively slow to reflect changes in diet (Hesslein et al. 1993).

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Our search for a "tissue" that might respond faster led us to consider epidermal mucus. Fish mucus consists primarily of long chains of glycoprotcins (Shephard 1994). Mucus serves a host of functions in fish (e.g., disease resistance, osmotic and ionic regulation, defense against abrasion), and its composition may vary by species, sex, life history stage, and health (Shephard 1994). An indication of how rapidly ingested material may appear in fish mucus was provided by Horsberg and Høy (1991), who found that following a single oral dose, diflubenzuron (a chitin synthesis inhibitor) appeared in the mucus of Atlantic salmon (*Salmo salar*) within 12 h and peaked in 2 days.

The first stable isotope analysis of mucus of any animal was reported for earthworms by Schmidt et al. (1999) and further explored by Dyckmans et al. (2005). In both cases, those researchers found significantly faster turnover in mucus than in muscle tissue, with mucus turnover being modeled more satisfactorily by a two-pool model. To better understand the rates of change of stable isotopes in fish mucus compared with rates for muscle tissue, we conducted controlled studies of diet switching in hatchery-raised fish. We know of no prior reports of stable isotope analysis of mucus in fish.

Materials and methods

Sampling

Steelhead (sea-run rainbow trout, *Oncorhynchus mykiss*) were grown from fertilized eggs from multiple adult steelhead obtained from the Alsea River Hatchery, Alsea, Oregon, USA. Fry were placed in tanks at the Oregon Hatchery Research Center, Alsea, Oregon for feeding at 90 days after fertilization (17 May 2006; mean wet weight ± standard error (SE) = 0.24 ± 0.007 g; mean fork length ± SE = 31.3 ± 0.37 mm; n = 30). Feed to initiate growth was #0 Moore Clark Nutra Starter ($\delta^{15}N$ = 8.3‰ ± 0.07‰ SE; $\delta^{13}C$ = –19.9‰ ± 0.05‰ SE; n = 5), a fish-meal-based diet. At 2 months (17 July 2006; mean wet weight ± SE = 2.63 ± 0.43 g; mean fork length ± SE = 61.0 ± 2.67 mm; n = 10), we switched the fish to a 1 mm feed formulated to be predominantly plant-based, with low delta values for $\delta^{15}N$ (2.8‰ ± 0.01‰ SE and $\delta^{13}C$ (–23.2‰ ± 0.01‰ SE; n = 8). Fish grew on this feed until the start of the experimental diet switch (31 October 2006; mean wet weight ± SE = 18.4 ± 1.72 g; mean fork length ± SE = 116.3 ± 4.96 mm; n = 20) to a 1 mm feed formulated with fish meal to have higher delta values ($\delta^{15}N$ = 13.3‰ ± 0.02‰ SE; $\delta^{13}C$ = –18.1‰ ± 0.01‰ SE; n = 8 at experiment start; $\delta^{15}N$ = 13.4‰ ± 0.02‰ SE; $\delta^{13}C$ = –18.0‰ ± 0.01‰ SE; n = 7 at experiment end). We maintained the fish on this diet for the remainder of the experiment.

Fish (n = 10) were sacrificed twice a day for the first 4 days of the diet switch, daily for the next week, and approximately weekly thereafter until 5 March 2007 (mean wet weight ± SE = 46.1 ± 2.44 g; mean fork length ± SE = 168.1 ± 2.51 mm; n = 10). On 26 June 2007, we sampled one fish (wet weight = 88.2 g, fork length = 204 mm) for mucus by placing it in a plastic bag, lightly rubbing it for 20 s, then replacing it in its rearing tank (a prior survival experiment with 15 steelhead (R. Church, unpublished data) had established that such sampling could be done without affecting either short-term or long-term survival). One week later, we sacrificed this fish for muscle tissue analysis. This fish was not fed during the interim period. All samples were frozen immediately in individual plastic bags then transported to the laboratory on ice and maintained frozen (–20 °C) until tissue preparation for stable isotope analysis.

We collected epidermal mucus from individual sacrificed fish by the following procedure. Each fish was thawed at room temperature for 5 min. We lightly scraped mucus from the dorsal surface of each fish with a spatula, transferred the scrapings to a 20 mL glass scintillation vial, then added three successive 5 mL washings of reverse osmosis (RO) water to the vial, mixing between washings. We filtered the solution through a polycarbonate filter (5 μm) into a 50 mL glass vial to remove any scales or debris and then added 5 mL RO water through the filter as a final rinse. For the single sample taken by the nonlethal bag technique, we added 20 mL of RO water to the bag, rinsed the bag by shaking it, and then decanted the solution and filtered it as above. We froze the resultant filtrate solutions overnight, then freeze-dried them for 48 h. The resultant material was mixed by spatula prior to weighing for isotopic analysis.

For muscle tissue analyses, we removed 0.5–5 g (depending on size of the fish) of dorsal muscle anterior to the dorsal fin from each fish, freeze-dried it for 48 h, then ground it to a fine powder with a ball and mill grinder prior to weighing.

Isotopic analysis

Isotopic analysis was performed on approximately 1 mg (weighed to 0.001 mg) of the prepared muscle and mucus material using a Delta XP isotope-ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Costech ECS 4010 elemental analyzer with zero blank autosampler.

Isotope ratios were expressed as in units of per mil (‰) by

$$(1) \qquad \delta X = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] \times 1000$$

where X = ^{15}N or ^{13}C, R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$, and atmospheric nitrogen and Pee-Dee Belemnite carbonate are the standards (0‰). Overall analytical precision (standard deviation) for our analyses based on repeated measures of an internal standard of adult steelhead carcass ($\delta^{15}N$ = 11.5‰, $\delta^{13}C$ = –20.2‰; n = 17), replicate samples (n = 21 pairs), and four secondary working standards (n = 68) analyzed with our samples was within ±0.1‰ for both $\delta^{15}N$ and $\delta^{13}C$. We lipid-adjusted $\delta^{13}C$ results for muscle tissue (mean C:N ± SE = 3.59 ± 0.03, mean of adjustments ± SE = 0.23‰ ± 0.03‰, n = 118) and also for the fish meal (diet switch) feed (mean C:N ± SE = 6.46 ± 0.01, mean of adjustments ± SE = 3.08‰ ± 0.01‰, n = 15) using C:N ratios according to the approach and recommendations of Post et al. (2007). We did not lipid-adjust $\delta^{13}C$ results for mucus samples (mean C:N ± SE = 3.41 ± 0.02, n = 118), as glycoproteins dominate the composition of mucus (together with a lesser mix of nonlipid components) (Shephard 1994).

Rates of change

Immediately after the diet switch (31 October 2006), there was a generalized cessation of feeding by the fish for approximately 1 week as the fish became accustomed to the

Fig. 1. (*a*) The $\delta^{15}N$ values of steelhead (*Oncorhynchus mykiss*) following initiation of switching from a feed with low $\delta^{15}N$ (2.8‰) to a feed with higher $\delta^{15}N$ (13.3‰). (*b*) The $\delta^{13}C$ values of steelhead following initiation of switching from a feed with low $\delta^{13}C$ (–23.2‰) to a feed with higher $\delta^{13}C$ (–18.1‰). Open circles are muscle tissue; solid diamonds are mucus. Solid lines are model fits based on the approach (eq. 2 this text) of Hesslein et al. (1993).

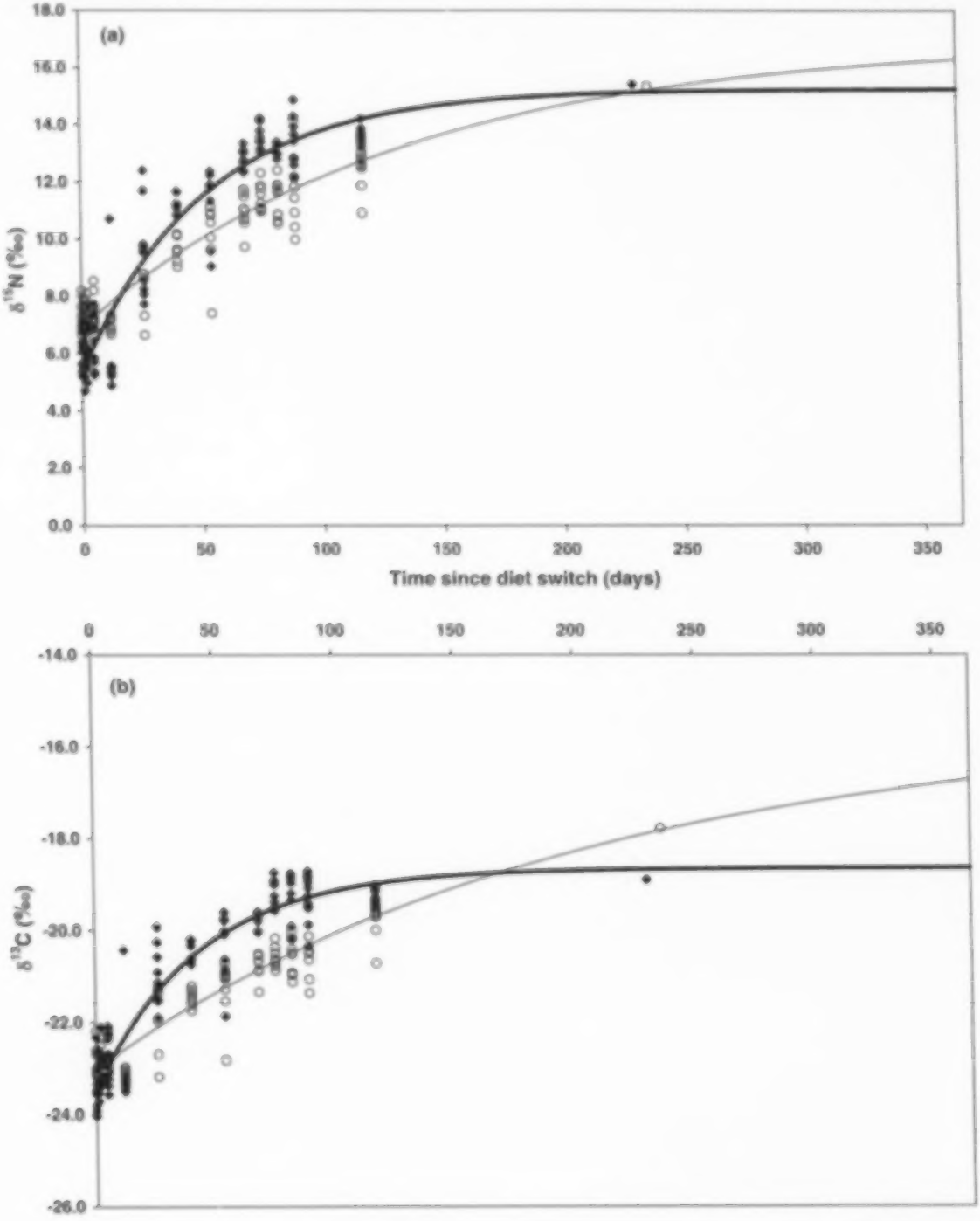

Table 1. Results of model fits for the diet switch experiment.

	Muscle ^{15}N	Mucus ^{15}N	Muscle ^{13}C	Mucus ^{13}C
Rates of change				
Coefficient of isotopic change growth (k) + turnover (m) (day^{-1}) (SE)	0.0074a (0.0017)***	0.0192b (0.0025)***	0.0051a (0.0015)*	0.0229b (0.0030)***
Isotopic change half-life, $t_{1/2}$ (days) (range)	94 (76–122)	36 (32–42)	136 (105–193)	30 (27–35)
Growth (k) (day^{-1}) (SE)	0.0065a (0.0004)***	NA	0.0065a (0.0004)***	NA
Fractionation				
$\delta_{initial}$ (‰) (SE)	6.9 (0.1)***	5.4 (0.2)***	−23.0 (0.1)***	−23.5 (0.1)***
δ_{final} (‰) (SE)	17.0 (1.6)***	15.3 (0.7)***	−15.6 (1.7)**	−18.7 (0.3)***
δ_{food} (‰) (SE)	13.3 (0.02)	13.3 (0.02)	−18.1 (0.01)	−18.1 (0.01)
$\delta_{final} - \delta_{food}$ (‰) (SE)	3.6 (1.6)	1.9 (0.7)	2.5 (1.7)	−0.6 (0.4)

Note: Significance levels for modeled curve fitting are shown: ***, $P < 0.0001$; **, $P < 0.001$; *, $P < 0.01$. Half-life $t_{1/2} = \ln(2)/(k + m)$. Different letters (a, b) indicate significant difference for a two-sample t test ($\alpha = 0.05$). Growth was determined from weights of individual fish according to $W_t = W_0 e^{kt}$ ($r^2 = 0.91$, $P < 0.0001$). SE, standard error; NA, not applicable.

new diet. This hiatus in feeding was apparent as the fish, which had been growing in an exponential fashion (R. Church, unpublished data), immediately ceased to grow. Based on observed feeding and growth patterns, we selected 8 November 2006 as the date of the switch to the new diet for analyzing the population rates of change of isotopic ratios for mucus and muscle tissues. We excluded six fish (5% of the fish analyzed) from the early stages of the analysis of rates of change because it was apparent from the isotopic analysis that prior to the diet switch they had been consuming either small live fish from the population or, as occasionally observed, parts of fish that had perished and had not yet been removed from the tank.

We modeled changes in $\delta^{15}N$ and $\delta^{13}C$ for both muscle and mucus using the equation of Hesslein et al. (1993):

$$(2) \qquad \delta_t = \delta_{final} + (\delta_{initial} - \delta_{final})\, e^{-(k+m)t}$$

where δ_t is $\delta^{15}N$ or $\delta^{13}C$ at time t, $\delta_{initial}$ is the delta value at the start of the analysis, δ_{final} is the delta value at equilibrium with the new diet, k is the coefficient of growth, and m is the coefficient of metabolic turnover. We determined $\delta_{initial}$, δ_{final}, and $(k + m)$ using the three-parameter exponential rise-to-maximum formulation of SigmaPlot graphical software (version 10.0, Systat Software Inc., Chicago, Illinois). We determined k from weights of individual fish fit to the equation

$$(3) \qquad W_t = W_0\, e^{kt}$$

where W_t is the weight at time t, and W_0 is the initial weight.

Results and discussion

Once fish began feeding, isotopic ratios rose for both muscle tissue and mucus in an exponential manner consistent with eq. 2. Isotopic ratios responded faster for fish mucus than for fish muscle for both $\delta^{15}N$ and $\delta^{13}C$ (Figs. 1*a* and 1*b*, Table 1). The lumped coefficients $(k + m)$ for muscle $\delta^{15}N$ and $\delta^{13}C$ were not significantly different from each other. The coefficients for mucus $\delta^{15}N$ and $\delta^{13}C$ also did not significantly differ from each other. Computed half-lives of response (Table 1) are a convenient metric for comparing the rates of response and indicate the relative rapidity of response of fish epidermal mucus relative to muscle. Half-lives for muscle tissue were 94 days for $\delta^{15}N$ and 136 days for $\delta^{13}C$. In comparison, the half-lives for isotopic change of mucus were 36 days for $\delta^{15}N$ and 30 days for $\delta^{13}C$.

The coefficient of growth (k, day^{-1}), as computed from fish weights (eq. 3), was 0.0065 (SE = 0.0004) and was not significantly different from that of the muscle tissue lumped parameter $(k + m)$ for either $\delta^{15}N$ or $\delta^{13}C$, thus precluding a meaningful calculation of a metabolic turnover rate (m) for muscle tissue. The comparison indicates that growth during this experiment dominated the change in isotopic ratios of muscle tissue. This is not surprising given the age and growth stage of the fish used here.

Our analyses provide estimates of δ_{final} for muscle and mucus for both $\delta^{15}N$ and $\delta^{13}C$ and thus for the computation of fractionation at equilibrium with the new diet (Table 1). The fractionations for muscle $\delta^{15}N$ and $\delta^{13}C$ are both quite similar to values reported by McCutchan et al. (2003) for hatchery-reared *O. mykiss* and *Salvelinus fontinalis*. Such values have been computed and reported numerous times for muscle tissue (e.g., McCutchan et al. 2003), but this is the first time that it has been determined for fish mucus. The companion equilibrium tissue differences for $(\delta_{muscle} - \delta_{mucus})$ also may be calculated readily from our results.

The coefficient of isotopic change for mucus (Table 1) is a new entity in stable isotope analyses of fish. Mucus is a small pool (as opposed to muscle) that is continually being synthesized, excreted, and then lost from the skin surface. Changes in isotopic ratios within mucus likely are a function of a shifting balance in synthesis between two primary pools — newly ingested feed and recycled peptides and amino acids from catabolized tissues (predominantly muscle). Consequently, the isotopic ratios for mucus will depend on isotopic ratios in both newly ingested feed and recycled tissues, as well as on attendant suites of fractionations during breakdown and synthesis. Examinations of concurrent patterns of changes in isotopic ratios of mucus and other tissues (e.g., muscle, liver) could shed new light on questions of tissue turnover in fish as a function of life stage and growth rate, a subject of consid-

erable interest (e.g., Herzka 2005). In addition, multiple tissues with different turnover rates can be effectively used in identifying diet shifts and their timing (Phillips and Eldridge 2006).

We have reported here the first instance of the use of fish mucus to quantify diet switching by fish. In our experiment, the rates of change for mucus were substantially faster than those of muscle tissues, even with our actively growing fish. With appropriately timed sampling designs, stable isotope ratio analysis of mucus could be of benefit in determining diet switching in wild populations where the duration of the shift is short and (or) the growth rates of the target organisms are low (Hesslein et al. 1993; MacAvoy et al. 2001). There are additional benefits where mucus may be collected nonlethally by the bag method we have described, especially for threatened or endangered species. Nonlethal collections of mucus may be combined with nonlethal fin clips to provide information on switches in diets and (or) habitats over both short and long time scales, respectively, without compromising sensitive populations.

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Relationship between spat density, food availability, and growth of spawners in cultured *Mizuhopecten yessoensis* in Funka Bay: concurrence with El Niño Southern Oscillation

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Abstract: To elucidate the factors that influence the interannual variation in the density of cultured Japanese scallop (*Mizuhopecten yessoensis*) spat, we analyzed the relationship between spat density (D_s), monthly chlorophyll *a* (chl *a*) concentration, water temperature, and adductor muscle weight of spawners over 15 years (1992–2006) in Funka Bay (Japan) on the western North Pacific Ocean. The interannual variation of spat density was best explained by a campaniform model that used chl *a* concentration in February ($[chl]_{F,ch}$) and a categorical variable that indicates whether growth of spawners in a year is low or not as independent variables ($R^2 = 0.91$). The gonadosomatic index increased fastest in February. Low growth years were detected as outliers in the D_s – $[chl]_{F,ch}$ relationship and were characterized by an average weight of adductor muscles in February of <12 g. Therefore, food availability during gonadal development and growth conditions of spawners were the main factors determining spat density. The proportion of ovary necrosis was high in the years of low $[chl]_{F,ch}$ and low growth. Those years corresponded with El Niño and La Niña years, respectively. Thus, global climatic anomalies apparently affect reproduction of the scallop in Funka Bay.

Résumé : Afin d'élucider les facteurs qui influencent la variation interannuelle de la densité du naissain des pétoncles du Japon (*Mizuhopecten yessoensis*) cultivés, nous avons analysé les relations entre la densité du naissain (D_s), la concentration mensuelle de la chlorophylle *a* (chl *a*), la température de l'eau et la masse du muscle adducteur des reproducteurs sur une période de 15 années (1992–2006) dans la baie de Funka (Japon) dans l'ouest du Pacifique Nord. La variation interannuelle de la densité du naissain s'explique le mieux par un modèle campaniforme qui utilise comme variables indépendantes la concentration de chl *a* en février ($[chl]_{F,ch}$) et une variable nominale qui indique si la croissance des reproducteurs est faible ou non pour une année donnée ($R^2 = 0.91$). L'indice gonadosomatique croît le plus rapidement en février. Les années de croissance lente se retrouvent comme valeurs aberrantes dans la relation D_s – $[chl]_{F,ch}$ et se caractérisent par une masse moyenne des muscles adducteurs <12 g en février. Ainsi, la disponibilité de nourriture durant le développement des gonades et les conditions de croissance des reproducteurs sont les facteurs principaux qui déterminent la densité du naissain. Le pourcentage de nécrose ovarienne est élevé les années de $[chl]_{F,ch}$ et de croissance faibles. Ces années correspondent respectivement aux années d'El Niño et de La Niña. Ainsi, les anomalies climatiques globales semblent affecter la reproduction des pétoncles de la baie de Funka.

[Traduit par la Rédaction]

Introduction

Interannual variations in recruitment are usually very large among marine soft-sediment benthos. To elucidate the influential factors, many studies have focused on larval supply (e.g., Butman 1987) and presettlement versus postsettlement processes (e.g., Olfsson et al. 1994). In bivalves, it is clear from laboratory studies that nutritional condition of the broodstock influences larval growth and survival (Barber and Blake 2006), but few field studies have demonstrated that nutritional condition of spawners influences recruitment. Food supply may be more critical to the nutritional condition of bivalves when they are cultured at greater densities than normally encountered in the wild, especially during the periods of gonad development when energy requirements are high (Barber and Blake 2006).

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Aquaculture of some bivalves, such as *Mizuhopecten yessoensis* (syn. *Patinopecten yessoensis*) in Japan and *Argopecten purpuratus* in Chile, starts with spat collection in natural conditions (Brand et al. 2006; Kosaka and Ito 2006). Data on larval supply of these species have been accumulated for a long time by many investigators, because information about spawning and the occurrence of planktonic larvae is required for effective spat collection in every year. Aquaculture of the Japanese scallop *M. yessoensis* began in the late 1960s in Funka Bay (42°22′N, 140°37′E). The annual production gradually increased from 11 500 tonnes (t) in 1980 to 108 000 t in 1989 (Hokkaido Prefectural Government 1982–2007). An extraordinarily low spat density was first reported in 1992 and has occurred in 5 of the past 15 years (1992–2006). In such years, producers must purchase seed from other areas at high cost.

Global climatic anomalies influence marine and terrestrial ecosystems worldwide (Stenseth et al. 2002). The direct influence of the El Niño – Southern Oscillation (ENSO) on the reproduction of marine bivalves in tropical and subtropical areas of the Pacific Ocean was reported (Urban and Tarazona 1996; Cantillanez et al. 2005; Riascos 2006). Because ENSO usually triggers the most dramatic changes in meteorological and oceanographic conditions over the entire North Pacific (Goes et al. 2001), ENSO should also influence bivalve reproduction because it probably affects ambient water temperature and food availability. However the influence of ENSO on bivalve reproduction has not been reported from temperate, subarctic, and arctic areas of the North Pacific.

The aim of this study is to elucidate direct environmental factors that influence the spat density in scallop culture in Funka Bay and develop a model to forecast spat density. We demonstrate that the nutritional condition of spawners influences recruitment and show that global climatic anomalies act on environmental factors. Our findings provide new viewpoints on factors influencing scallop recruitment and on the biological influences of ENSO because there are no reports that clearly link scallop recruitment to ENSO in high latitude areas of the North Pacific.

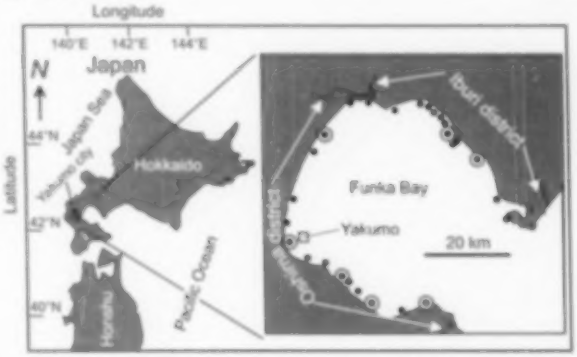
Materials and methods

Spat density, larval density, and spawning biomass

Spat density was measured as the number of spats per 100 g collector. Procedures for the collection of *M. yessoensis* spats are described in Kosaka and Ito (2006). To investigate the interannual variation of spat density in the Oshima district, we set two to four (usually three) spat collectors at each of three sites in 1992 and at each of five sites from 1993 to 2006 (Fig. 1; Table 1). In the Iburi district, single spat collectors were set at one site from 1995 to 2000 and at two sites from 2001 to 2006 (Fig. 1; Table 1). The collectors were set for 30–40 days from late May to early July. Samples were fixed in 5% formalin–seawater, diluted 1:2 one to five times, and counted under a profile projector with 50× magnification.

We measured the gonadosomatic index (GSI: *G*/BW, where *G* is gonad weight and BW is total body weight excluding shell) weekly during April and May near the sites of spat collection (Fig. 1). After the GSI decreased substantially, planktonic larvae were sampled weekly at 15 sites in Oshima and 12 sites in Iburi from four to nine times (Fig. 1; Table 2). Larvae were collected in a 300 mm diameter plankton net (NXX-13, 95 μm mesh) hauled vertically from a depth of 15 m to the surface. The filtration rate of the plankton net was considered to be 100%. Planktonic larvae of *M. yessoensis* were counted under a profile projector. Larvae were identified according to Nishikawa (1971). The density of the planktonic larvae was calculated as the number of larvae per cubic metre of seawater.

Fig. 1. Location of Funka Bay and sampling sites: ○, sampling sites for spat density; ●, sampling sites for planktonic larvae; □, measurement site for environment variables and growth of spawners at Yakumo.



The spawning season of Japanese scallops usually starts in late April in Funka Bay. The spawners are age-1 class, because almost all scallops are harvested before the spawning season of age 2; the scallop sexually matures at age 1, and age-0 scallops are thought to be immature (Kosaka and Ito 2006). The biomass of spawners can be roughly estimated from the harvest between May and December. To explore the relationship between the spawning biomass and spat and larval densities, we used the scallop harvest between May and December of each year from 1992 to 2006 (Hokkaido Prefectural Government 1982–2007) as an index of spawner biomass.

Spearman's rank correlation analysis was used to evaluate the relationship between spat density and maximum mean density of planktonic larvae within the Oshima district and to test for correlated changes in either variable between the Oshima and Iburi districts. Spearman's rank correlation analysis was also used to evaluate the relationships between the biomass of spawners and the spat density and the maximum larval mean density. Spat densities and maximum larval mean densities between the two districts were compared by Wilcoxon's signed-rank test. We compared slopes of regression lines expressing the relationship between spat density and larval density in high versus low spat density years by a *t* test according to Zar (1999).

Water temperature, chlorophyll *a* concentration, growth and reproductive conditions of spawners, and proportion of ovary necrosis

Water temperature (°C) and chl *a* concentration ($\mu g \cdot L^{-1}$) were measured monthly at 5 km off Yakumo (depth 32 m) for 15 years (1992–2006) (Fig. 1). The temperature was

Table 1. Spat density (D_s, number of spats per 100 g collector) in scallop culture in Funka Bay.

	Oshima				Iburi			
	$\log_{10}(D_s)$				$\log_{10}(D_s)$			
Year	Mean	SD	n_s	n_c	Mean	SD	n_s	n_c
1992	2.27	0.58	3	8				
1993	2.57	0.36	5	15				
1994	4.87	0.52	5	16				
1995	4.45	0.61	5	14	5.75		1	1
1996	4.40	0.34	5	15	4.93		1	1
1997	3.27	0.29	5	15	4.23		1	1
1998	1.48	0.62	5	15	2.26		1	1
1999	2.84	0.84	5	14	2.59		1	1
2000	3.00	0.21	5	14	3.26		1	1
2001	3.60	0.25	5	14	4.09	0.20	2	2
2002	3.00	0.40	5	15	3.73	0.07	2	2
2003	4.40	0.29	5	15	5.40	0.05	2	2
2004	4.66	0.17	5	15	5.34	0.14	2	2
2005	4.07	0.18	5	17	4.16	0.04	2	2
2006	3.91	0.42	5	15	5.03	0.29	2	2

Note: n_s, number of sites; n_c, number of spat collectors across all sites; SD, standard deviations.

Table 2. Maximum mean density of planktonic larvae of *Mizuhopecten yessoensis* (D_l, number of larvae per 1 m^3) in Funka Bay.

	Oshima			Iburi		
	$\log_{10}(D_l)$			$\log_{10}(D_l)$		
Year	Mean	SD	n	Mean	SD	n
1992	1.92	0.47	15	1.88	0.33	12
1993	2.35	0.34	15	1.82	0.48	12
1994	3.48	0.10	15	3.70	0.36	12
1995	3.18	0.24	15	3.40	0.23	12
1996	3.34	0.36	15	3.19	0.37	12
1997	2.50	0.50	15	2.28	0.22	12
1998	1.54	0.41	15	2.06	0.31	12
1999	2.84	0.17	15	2.71	0.35	12
2000	2.72	0.32	15	2.30	0.35	12
2001	2.50	0.30	15	2.60	0.22	12
2002	2.48	0.43	15	2.44	0.27	12
2003	2.69	0.29	15	3.04	0.29	12
2004	3.57	0.25	15	3.89	0.19	12
2005	2.35	0.18	15	2.93	0.21	12
2006	2.73	0.52	15	3.28	0.16	12

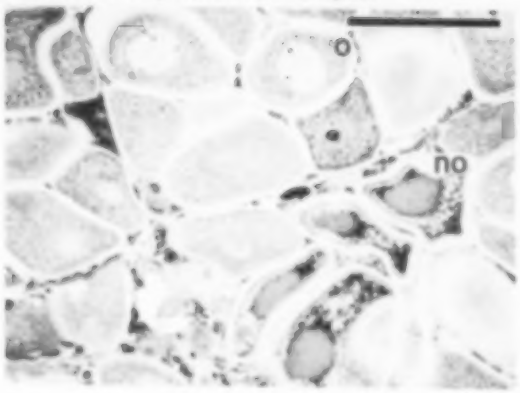
Note: SD, standard deviation; n, number of sites.

measured at every 1 m depth by conductivity–temperature–depth recorder (CTD; SBE 19–01, Seabird Electronics Inc., Bellevue, Washington, USA). For measurement of chl *a*, 300 mL of seawater was sampled by a Bandon water sampler at depths of 5, 10, and 15 m. Each sample was filtered by glass microfiber filter (CF/F, 47 mm, Whatman International Ltd, Maidstone, England) and chl *a* was extracted with 10 mL of DMF (*N*,*N*-dimethylformamide). The content of chl *a* was measured from the change in fluorescence (excitation 436 nm, emission 660 nm) before and after acidification by adding 0.1 mL of 0.6 mol·L^{-1} HCl in 4 mL of the sample DMF solution. Fluorescence was measured by a fluorescence spectrophotometer. The concentrations of chl *a* were calculated by using chl *a* from spinach (Sigma, St. Louis, Missouri, USA) as a standard. We used averages of the temperature and concentration of chl *a* from 5 to 15 m for further analyses because scallops are usually grown at these depths.

To investigate interannual and seasonal changes in the growth and maturity of the spawners, we sampled age-1 scallops (settled from the previous July to the current-year May) monthly at a culture facility near the site of environmental measurements. Age-1 scallops were cultured by the ear-suspend method on 13 m ropes. Ten scallops were sampled from each of the upper, middle, and lower parts of the rope (total 30 scallops). Adductor muscle, gonads, and the other viscera of each individual were weighed, and the GSI was estimated. The adductor muscle is an important organ for the storage of glycogen used for gametogenesis (Barber 1984). GSI is commonly used to determine the reproductive season and to assess the allocation of energy to reproduction (Urban and Riascos 2002).

To derive a physiological index of spawner condition, we calculated the proportion of ovary necrosis in early to mid-April at Yakumo over seven years (2000–2006). Necrotic oocytes are characterized by changes to their staining affinities, beginning with the nucleus, which loses its basophilic properties (nuclei of necrotic oocytes are strongly stained by eosin, but normal ones are less stained), and they become very deformed (Beninger and Le Pennec 2006) (Fig. 2). To evaluate the extent of ovary necrosis, we examined the ovaries of 10 age-1 individuals in each year. The middle part of each ovary, 5 mm thick, was dissected out and fixed in 4% paraformaldehyde in 0.1 mol·L^{-1} phosphate buffer (pH 7.4) overnight. The fixed samples were dehydrated through a graded ethanol series, embedded in paraffin, and sectioned at 4 μm. After removal of the paraffin, sections were stained with Mayer's hematoxylin and eosin. To evaluate the proportion of each ovary that was necrosed, we took photographs at six sites in each section, each covering 628 μm × 443 μm. The outlines of the necrotic areas were traced by

Fig. 2. Ovary of Japanese scallop *Mizuhopecten yessoensis*. o, oocyte; no, necrotic oocyte (scale bar = 100 μm).



hand on a transparent sheet placed over each photograph. The profiles were digitized with a scanner, and the areas were measured by an image analysis program (Scion Image; Scion, Frederick, Maryland, USA). The necrosed proportion was calculated as NR = NA/PA, where NA is the sum of the necrotic areas in the six photographs and PA is the area covered by the photographs. We tested significance of interannual variation of the proportion of ovary necrosis by the Kruskal–Wallis test. We evaluated the relationship between the proportion of ovary necrosis and spat density by Spearman's rank correlation. To check for histological anomaly in testes, we also observed testes from 10 males sampled in April 2000.

Modeling of spat density

Variables, models, and fit

We analyzed data from the Oshima district for three reasons: the environmental measurements were taken and the spawners grew in the middle of Oshima (Fig. 1); data on spat density have been collected for longer in Oshima than in Iburi (Table 1); and 79% to 87% of total scallops (weight) produced in Funka Bay were harvested in the Oshima district during 1992 to 2003 (Hokkaido Prefectural Government 1982–2007). We log-transformed spat densities to avoid multiplicative effects and heteroscedasticity (Sokal and Rohlf 1995).

As independent variables, we used monthly water temperatures and chl *a* concentrations (from the previous July to the June of the spat-collection year, i.e., 24 variables) and a categorical variable that indicates whether a year is an outlier or not, and combinations of these variables. The number of parameters should be less than or equal to $2n^{1/2}$ or $n/2$, where n is sample size (Sakamoto et al. 1983). Because the sample size is 15 and our models have three constant terms (including a random error term), we should not use more than four independent variables. The numbers of models that we tested were $\sum_{i=1}^{4} C_i^{24} = 12\,950$ without the categorical variable and $\sum_{i=1}^{4} C_i^{25} = 15\,275$ with the categorical variable (total, 28 225 models).

The sigmoid and campaniform models were modified from probability distribution and probability density functions, respectively, of a multivariate logistic distribution. The sigmoid model (eq. 1) and campaniform model (eq. 2) are

$$(1)\quad f(x_j) = a_{\max}\left\{1 + \exp\left[-\left(a_0 + \sum_{j=1}^{n} a_j x_j\right)\right]\right\}^{-1}$$

and

$$(2)\quad f(x_j) = a_{\max}\exp\left[-\left(a_0 + \sum_{j=1}^{n} a_j x_j\right)\right] \times\left\{1 + \exp\left[-\left(a_0 + \sum_{j=1}^{n} a_j x_j\right)\right]\right\}^{-2}$$

where $f(x_j)$ is the predicted spat density, n_i is the number of independent variables, x_j is the jth independent variable, and $a_{\max}$, a_0, and a_j are coefficients.

To fit the models for model selection, we used the maximum likelihood method on the assumption of a normal distribution with random error distribution. The log-likelihood function to estimate the coefficients of eqs. 1 and 2 is

$$(3)\quad \log_e L(a_{\max}, a_0, a_j, \sigma) = \sum_{i=1}^{n} \log_e\left\langle \frac{1}{\sigma(2\pi)^{1/2}}\exp\left\{\frac{-[y_i - f(x_{i,j})]^2}{2\sigma^2}\right\}\right\rangle$$

where y_i is the observed spat density in year i, $f(x_{i,j})$ is the predicted spat density of year i, n is sample size, and σ is the standard deviation (scale parameter) of random error. The coefficients $a_{\max}$, a_0, a_j, and σ are estimated as values that maximize the log-likelihood function.

To detect candidate outliers, we fitted models by the maximum likelihood method on the assumption of a Cauchy distribution with random error distribution. The Cauchy distribution is robust against outliers (Press et al. 1992), i.e., candidates are presumed to be detected. The log-likelihood function is

$$(4)\quad \log_e L(a_{\max}, a_0, a_j, b) = \sum_{i=1}^{n} \log_e\left\langle \pi b\left\{1 + \left[\frac{y_i - f(x_{i,j})}{b}\right]^2\right\}\right\rangle^{-1}$$

where b is the scale parameter of the Cauchy distribution. The coefficients $a_{\max}$, a_0, a_j, and b are estimated as values that maximize the log-likelihood function.

Model selection

To compare models, we calculated AIC_c, a statistical criterion modified from AIC (Akaike's information criterion; Akaike 1973) by Hurvich and Tsai (1995) for empirical correction for small sample size. Burnham and Anderson (1998) suggested using AIC_c regardless of sample size because AIC_c converges to AIC as n gets large. AIC_c is calculated by

$$(5)\quad AIC_c = -2\log_e L + 2K + \frac{2K(K+1)}{(n-K-1)}$$

where $\log_e L$ is the maximized value of log-likelihood, n is sample size, and K is the number of estimable parameters. The model that has the minimum AIC_c is usually regarded as the best model.

To evaluate model selection uncertainties, we estimated the bootstrap selection probabilities ($\bar{\pi}_i$) by $\bar{\pi}_i = b_i/B$, where b_i is the number of replicates in which model i was selected as the minimum-AIC_c model, and B is the bootstrap replicates. We estimated $\bar{\pi}_i$ in the models that gave the minimum AIC_c among those that used the same number of parameters, because to estimate $\bar{\pi}_i$ among all models is very computer-intensive. In our analyses, both dependent and independent variables were averaged values of raw data. Hence, the procedures of the bootstrap were as follows. (*i*) The same number (as original data) of data were randomly sampled from original data (allowing sampling of same data twice or more) for each dependent and independent variable. (*ii*) Averages of the randomly sampled data were calculated. (*iii*) Using the averaged values, coefficients of models were optimized in the same manner as in the original analysis, and then AIC_cs were calculated. (*iv*) We repeated procedures *i* to *iii* B times and counted the replicates (b_i) in which model i was selected as the minimum-AIC_c model. We performed the bootstrap sampling 10 000 times (10 000 replicates).

To evaluate the uncertainties of the coefficients and R^2 and AIC_c, we estimated 95% confidence intervals by the bootstrap method simultaneously with the calculation of $\bar{\pi}_i$. The values in the upper and lower 2.5% of the 10 000 bootstrap estimates were regarded as the upper and lower 95% confidence limits.

Concurrence of El Niño and La Niña with years of low spat density and low spawner growth

To check concurrence of global climatic anomalies with spat density and growth condition of spawners, we investigated the duration of El Niño and La Niña (both by new and old definitions; Table 3) from the Japan Meteorological Agency (2007). We judged concurrence by whether or not each event was active in Funka Bay during spring (March–May) when *M. yessoensis* gonad development is completed and spawning occurs. We used a Monte Carlo simulation to test the significance of concurrences: El Niño with low spat density years, La Niña with low growth years of the spawners, and normal years with high spat density years (see results for these classifications). The null hypothesis was that any observed concurrence is coincidental. In our 15 years, El Niño was active in spring in three years (and two more years by the old definition of El Niño), and La Niña was active in spring in three years. In the Monte Carlo simulation, we randomly allocated three (or five) El Niño and three La Niña events to 15 years 10 000 000 times and calculated the probability (P) of the cases that showed values better than, or equal to, the observed concurrence. To check the precision of the probability, we carried out bootstrap resampling 10 000 times and regarded the values at the upper and lower 2.5% of the 10 000 estimates as the upper and lower 95% confidence limits, respectively.

To infer how El Niño and La Niña influenced the oceanographic conditions in Funka Bay, we investigated temperature (°C), sunshine (h·day^{-1}), precipitation (mm), snowfall (cm), and wind (m·s^{-1}) from daily AMeDAS data collected at Yakumo City in the middle of the coast of Funka Bay (from the homepage of the Japan Meteorological Agency, http://www.jma.go.jp/jma/indexe.html). Differences in low spat density years and years of low spawner growth relative to normal years (high spat density years) in each climate factor were evaluated by P of a t test with Welch's correction. We also checked the correspondences with characteristics of temperature, sunshine, and precipitation of El Niño and La Niña years in northern Japan presented by the Japan Meteorological Agency (2007) (the only characteristics presented).

Results

Interannual variation of spawner biomass, larval density, and spat density

The biomass of spawners, estimated from harvest between May and December during 15 years (1992–2006) ranged from 15 821 t (2004) to 45 765 t (2002). In Oshima district, spat density varied by a factor of >1000 and maximum larval mean density varied by a factor of >100 over the period 1992–2006 (Tables 1, 2). Spat density and the maximum mean density of planktonic larvae in each year were positively correlated ($r = 0.78$, $P < 0.001$). Spat densities during 1995–2006 were significantly correlated between Oshima and Iburi ($r = 0.85$, $P < 0.001$; Table 1). Maximum larval mean densities were also significantly correlated between Oshima and Iburi ($r = 0.92$, $P < 0.001$; Table 2). Larval densities were higher in Iburi than in Oshima ($P < 0.01$), whereas spat densities were not significantly different between the two districts ($P > 0.05$). The correlation between the biomass of spawners and the spat density was not significant ($r = -0.57$, $P = 0.58$; Fig. 3), nor was the correlation between spawning biomass and larval density ($r = -0.11$, $P = 0.70$; Fig. 3).

Relationship between spat density and food availability during gonad development and spawner growth

The concentration of chl *a* in February ($[chl]_{Feb}$) had the greatest influence on the interannual variation in spat density, as indicated by the smallest AIC_c among both sigmoid and campaniform single-variant models (Table 4). In the campaniform model using $[chl]_{Feb}$ as an independent variable, five candidate outliers were detected when the Cauchy distribution was assumed with random error (Fig. 4*b*), but they were not detected when the normal distribution was assumed with random error (Fig. 4*a*). We failed to detect apparent candidate outliers with the sigmoid model.

Three out of the five candidate outliers (3, 4, and 5 in Fig. 4*b*) correspond to years of very low growth of spawners. We defined these three years (1999, 2000, and 2006) as low growth years and treated them as actual outliers. These years took a value of 1 for the categorical variable that indicates whether a year is a low growth year or not (Gc) (other years took a value 0 for the categorical variable). Models were much improved in terms of AIC_c by adding this categorical variable (Table 4).

The campaniform model was better than the sigmoid model, as the total of bootstrap selection probabilities ($\bar{\pi}_i$) were 0.896 and 0.104, respectively. Model 4 was the best in terms of AIC_c; it was not significantly different from the AIC_c of models 2 or 3, thus showing substantial model-selection uncertainty. Model 4 had a bootstrap selection probability of only 0.567, and models 2 and 3 had probabilities of 0.159 and 0.169, respectively (Table 4). We selected the campaniform model 2 with two independent

Table 3. Definitions of El Niño and La Niña and durations of these events during the study period (Japan Meteorological Agency 2007).

	New definition (from April 2006)	Old definition
Area	5°N–5°S, 150–90°W (NINO.3 area)	4°N–4°S, 150–90°W (B area)
Index	Five months moving average of difference from the basis (monthly average of past 30 years) to current monthly average temperature of surface ocean water	Five months moving average of difference from the basis (monthly average during 1961–1990) to current monthly average temperature of surface ocean water
Conditions	+0.5 °C (El Niño), –0.5 °C (La Niña), for more than 6 months	+0.5 °C (El Niño), –0.5 °C (La Niña), for more than 6 months
Duration		
El Niño	1991 spring – 1992 summer	1991 spring – 1992 summer
		1993 spring – 1993 summer
	1997 spring – 1998 spring	1997 spring – 1998 summer
	2002 summer – 2002–2003 winter	2002 spring – 2002–2003 winter
La Niña	1995 summer – 1995–1996 winter	
	1998 summer – 2000 spring	1998 autumn – 1999 spring
		1999 summer – 2000 spring
	2005 autumn – 2006 spring	2005 autumn – 2006 spring

Note: Spring, March–May; summer, June–August; autumn, September–November; winter, December–February.

variables ($[chl]_{Feb}$ and the categorical variable) to best describe the relationship between spat density and both environmental influence and spawner condition. That is, we selected the simplest among the models with values of AIC_c that were not significantly different from that of the minimum-AIC_c model.

The campaniform model 2 gave a good fit to the spat densities (R^2 = 0.91; Fig. 5). Spat densities were low in the years in which $[chl]_{Feb}$ was low (1992, 1993, 1997, 1998, and 2002); we defined these five years as low spat density years. They were high in seven years in which $[chl]_{Feb}$ was high (1994, 1995, 1996, 2001, 2003, 2004, and 2005); we defined these seven years as high spat density years. The predicted value of spat density peaked at $[chl]_{Feb}$ = 5.6 μg·L^{-1}. Spat densities of years of low spawner growth were lower than predicted from $[chl]_{Feb}$ alone (Fig. 5). The coefficients of model 2 were reasonably estimated because the 95% confidence limits were not extraordinarily large and the value 0 was not included in any of the confidence intervals.

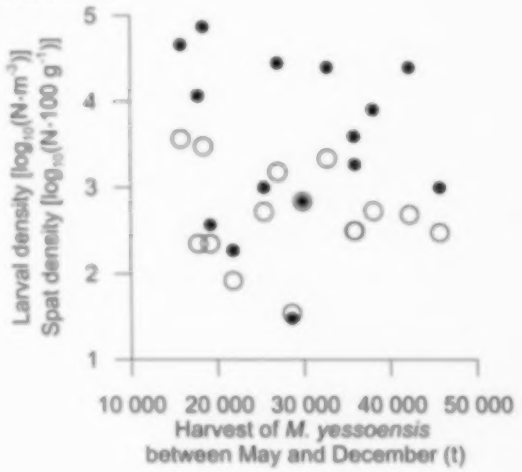
In terms of AIC_c, the best model (model 2) was not improved by adding the biomass of the spawners as an independent variable. The slopes of the relationships between spat density and maximum larval mean density were different between high and low spat density years ($P < 0.05$; Fig. 6).

Fastest rate of increase in GSI occurred in February (Fig. 7*d*). Adductor muscle weights of the spawners in the low growth years were very low, especially after August (Fig. 7*c*), and were the lowest during December to February over the period 1992–2006. The averages of adductor muscle weight in February of these three years were less than 12 g (mean ± SE: 11.5 ± 0.5 in 1999, 10.1 ± 0.7 in 2000, 11.1 ± 0.4 in 2006). Other than these three years, adductor muscle weights in February varied from 12.7 ± 0.3 g (2001) to 21.0 ± 0.3 g (1996).

Characteristics of water temperature, chlorophyll *a* concentration, and spawners in high and low spat density years and low growth years

Our CTD data indicated apparent vertical stratifications

Fig. 3. Relationship between harvest of *M. yessoensis* between May and December (index of spawning biomass) and spat (●) and larval (○) density.



that were usually established in April (but March in 1995, 2002, and 2004 and May in 2000 and 2006), mainly by the thermocline. Vertical stratifications are usually established by thermocline or halocline (Parsons et al. 1995). Winter and spring blooms started before the water column became stratified in all years, and maximum concentrations of chl *a* were recorded in March (except February in 1995) (Fig. 7*b*). We defined the autumn bloom as chl *a* enhancement initiated during August–September, the winter bloom as initiated during October–December, and the spring bloom as initiated during January–March. The duration of winter blooms and the time of initiation of spring blooms were significantly correlated in high and low spat density years (r = 0.67, P = 0.02, Spearman's rank correlation), i.e., spring blooms started earlier when winter blooms were shorter. In high spat density years, winter blooms were not observed or were observed in only one month. Except in 1995, spring blooms started in January when a winter bloom did not oc-

Table 4. Results of modeling of spat density (dependent variable), independent variables, coefficients, R^2, AIC_c, and bootstrap selection probability ($\hat{\pi}_i$) of models that have minimum AIC_c among models with the same number of independent variables.

		Constant terms			Independent variables									
No.	Model type	σ	θ_{max}	θ_0	$[Chl]_{Jan}$ (α_1)	Gc (α_2)	$[Chl]_{Nov}$ (α_3)	$[Chl]_{Apr}$ (α_4)	$[Chl]_{Nov}$ (α_5)	$[Chl]_{Aug}$ (α_6)	$[Chl]_{Oct}$ (α_7)	R^2	AIC_c (95% confidence interval)	$\hat{\pi}_i$
1	C	0.57	17.05	−1.77	0.27							0.64	38.0 (42.5–34.4)	0.000
2[a]	C	0.29	19.26	−2.02	0.36		−1.12					0.91	22.1 (32.9–18.1)	0.159
3	C	0.22	19.20	−1.64	0.34		−1.27	−0.17				0.95	20.0 (34.4–14.6)	0.169
4	C	0.12	19.25	−1.69	0.33		−1.34	−0.24	0.12			0.98	9.4 (39.3–6.9)	0.567
5	S	0.57	4.07	−0.42	1.04							0.64	37.8 (41.7–34.3)	0.000
6	S	0.36	4.38	−0.56	1.01		−3.25					0.86	28.4 (34.7–24.7)	0.038
7	S	0.28	4.33	0.04	1.47		−4.85			−0.10		0.92	26.7 (33.6–23.8)	0.066
8	S	0.26	4.38	0.97	1.50		−3.53				−2.83	0.93	32.1 (44.0–30.7)	0.000

Note: Model type: C, campaniform; S, sigmoid. Gc, categorical variable indicating whether a year is a low growth year (1) or not (0).
[a]The model ultimately selected.

Fig. 4. Campaniform models fitted to spat densities (number of spats per 100 g collector) with chlorophyll *a* concentration in February as an independent variable: (*a*) assuming normal distribution with random error distribution; (*b*) assuming Cauchy distribution with random error distribution. Solid line, mean of model prediction; broken lines, 90% confidence limits of random error; 1–5, candidate outliers.

cur (1994 and 1996) or in February when winter blooms were observed in only one month (2001, 2003, 2004, and 2005). In 1995, the winter bloom lasted one month (October) and the spring bloom started in January. In low spat density years except 1997, winter blooms were observed in two months (2002) or three months (1992, 1993, and 1998) and spring blooms started in March. In 1997, the winter bloom was not observed and the spring bloom started in March. In years of low spawner growth (1999, 2000, and 2006), the winter bloom was not observed and the spring bloom started in February. Autumn blooms (chl *a* enhancement during August–September) were observed in only four years, in one or two months (1992 in September; 1994 and 1999 in September–October; 2006 in August). In low spat density years, influenced by the profile of chl *a*, weights of adductor muscle were higher in January but were much lower in March than those in high spat density years (Fig. 7*c*); GSIs were also higher in January but much lower in March than in high spat density years (Fig. 7*d*).

Fig. 5. Relationships between spat density (number of spats per 100 g collector) and chlorophyll *a* concentration in February and low growth of spawners in scallop culture in Funka Bay. Bold lines indicate the predictions for years of low growth of spawners; light lines indicate the predictions for other years. Numbers near plots indicate year of data. Solid lines, means of model prediction; broken lines, 90% confidence limits of random error; ●, high spat density years; △, low spat density years; ○, low growth years; *, El Niño was active in the spring in Funka Bay; *1, El Niño was active in the spring according to the old definition of the Japan Meteorological Agency (2007); **, La Niña was active in the spring; bar = standard error, SE.

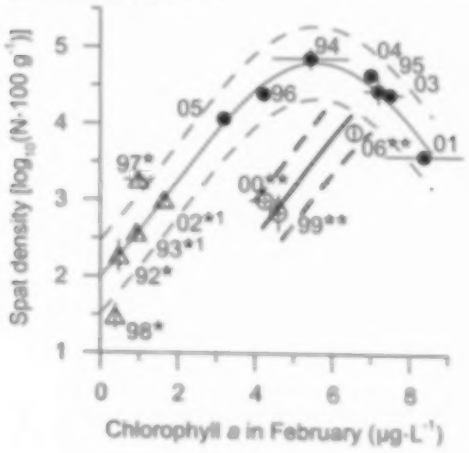


Fig. 6. Relationships between spat density and maximum larval mean density each year in Oshima, Funka Bay. Solid and broken lines are regression lines for years of abundant and low spat density, respectively: ●, high spat density years; △, low spat density years; ○, low growth years.

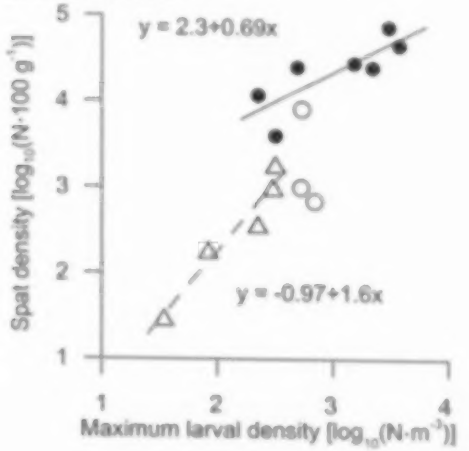


Fig. 7. Monthly changes of (*a*) temperature (°C), (*b*) chlorophyll *a* concentration (μg·L^{-1}), (*c*) adductor muscle weight (grams), and (*d*) gonad–somatic index of age-1 scallops (spawners) at Yakumo in Funka Bay: ●, high spat density years; △, low spat density years; ○, low growth years; bar = standard deviation, SD.

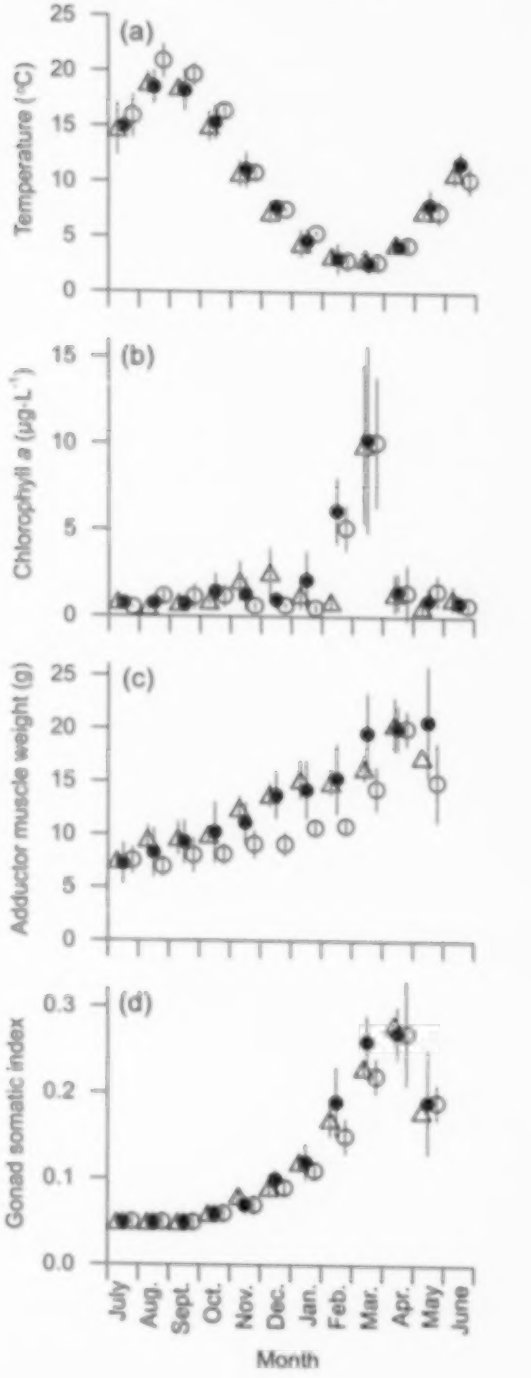


Table 5. Climatic differences in years of low spat density and low spawner growth from normal years (high spat density years) in Yakumo City based on AMeDAS data (Japan Meteorological Agency 2007).

	Low spat density years				Low growth years			
	Temperature	Sunshine	Snow	Wind	Temperature	Sunshine	Snow	Wind
Summer	– – –*	– –*			+ +*			+
Autumn							+ + +*	
Winter	+ + +*		– – –*	– – –			+*	– – –
Spring	+ +*	+*	– – –			– – –*		

Note: + (positive) and – (negative), $0.05 \leq P < 0.10$ by t test with Welch correction; + + and – –, $0.01 \leq P < 0.05$; + + + and – – –, $P < 0.01$; *, Result that corresponded with climatic characteristics in El Niño years and La Niña years in northern Japan (Japan Meteorological Agency 2007); Spring, March–May; summer, June–August; autumn, September–November; winter, December–February.

In years of low spawner growth, water temperatures were very high during August–October, and low chl *a* persisted during November–January (Figs. 7*a*, 7*b*).

Relationship between spat density and ovary necrosis and histological observation of testis

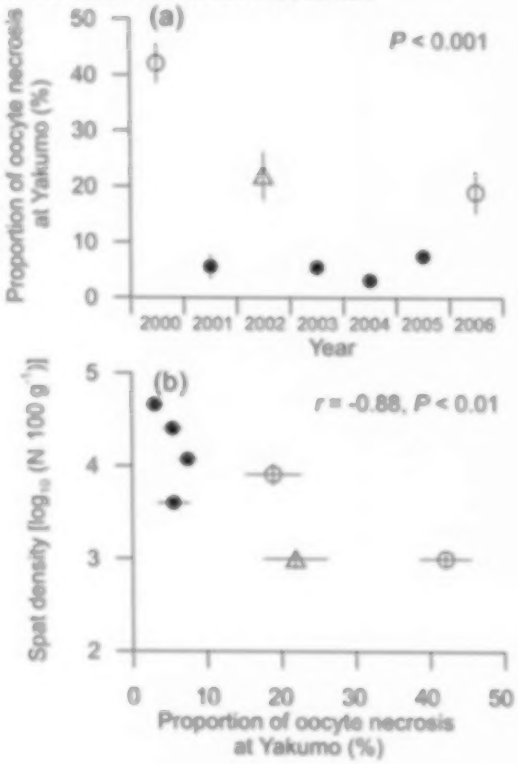
Proportions of ovary necrosis in April at Yakumo varied significantly among years ($P < 0.001$) and were higher in low spat density years and low growth years than in high spat density years (Fig. 8*a*). Spat density was negatively correlated with the proportion of necrosed ovary in Oshima ($r = -0.88$, $P < 0.01$; Fig. 8*b*). We found no anomaly in the testes.

Concurrence of El Niño and La Niña with years of low spat density and low growth of spawners

The concurrence of El Niño with years of low spat density, of La Niña with years of low spawner growth, and of normal years with high spat density was 15/15 = 1.00 by the old definition of El Niño and 13/15 = 0.87 by the new definition only (Fig. 5; Table 3). These concurrences were highly significant, according to the Monte Carlo simulations ($P = 4.0 \times 10^{-6}$ for concurrence = 1.00, 95% CI = 8×10^{-6} and 1.0×10^{-6}; $P = 8.6 \times 10^{-5}$ for concurrence = 0.87, 95% CI = 1.0×10^{-4} and 6.9×10^{-5}). In all five low spat density years (1992, 1993, 1997, 1998, and 2002), El Niño was active in spring in Funka Bay (by the old definition of El Niño in 1993 and 2002). In all three years of low spawner growth (1999, 2000, and 2006), La Niña was active in spring. In all seven high spat density years (1994, 1995, 1996, 2001, 2003, 2004, and 2005), neither El Niño nor La Niña was active in spring.

Climatic differences in years of low spat density years and of low spawner growth relative to normal years (high spat density years) were detected in the AMeDAS data (Table 5). These characteristics mostly corresponded with the climatic characteristics of El Niño and La Niña years presented by the Japan Meteorological Agency (2007). No significant difference was detected in the precipitation.

Fig. 8. (*a*) Proportion of ovary necrosis each year at Yakumo in Funka Bay (P value was calculated by Kruskal–Wallis test). (*b*) The relationship between the proportion of necrotic oocytes at Yakumo and the spat density, indicated as number of spats per 100 g collector, in Oshima (r and P values were calculated by Spearman's rank correlation). ●, high spat density years; △, low spat density years; ○, low growth years. Vertical bars in (*a*) and horizontal bars in (*b*) are standard deviations (SDs) of averages of necrosis proportion of individuals. Vertical bars in (*b*) are SDs.



Discussion

Relationships between spat density and food availability during gonadal development and growth of spawners

Food availability during gonadal development and growth conditions of spawners were the most important factors determining spat density, because a large part of the interannual variation in spat density could be explained by the concentration of chl *a* in February, when GSI increased fastest, and whether or not a year was one of low spawner growth. Scallops cannot allocate enough nutrients to gonadal development in years of low $[chl]_{Feb}$ or in years of low growth of spawners, so oocytes that cannot accumulate enough nutrients may die. Hence, the proportion of ovary

necrosis was high in such years. The variability about the mean proportion of ovary necrosis was much higher in the years of low growth and of low spat density than in the years of high spat density. This suggests that some individuals had healthy ovaries in years of low growth, whereas others did not. During 2000–2006, spat density (D_s) varied by a factor of 251 ($\log_{10}(D_s)$ = 3.00 in 2000 and 5.40 in 2003) and the proportion of necrosed ovary varied from a low of 3.1% in 2004 to a high of 42.1% in 2000. The interannual variation in spat density seemed very large relative to the proportion of necrosis in ovaries. We do not think that this divergence was caused by the small sample size for determining the extent of ovary necrosis in the spawning population. The sample size seemed to be enough to detect interannual changes and to describe physiological ovarian conditions because significant results were obtained in our analyses. In highly necrotized ovaries, the conditions might be worse than histological observations showed, because normal oocytes might be affected by lytic enzymes released throughout the acinus when other necrotic oocytes lyse (Beninger and Le Pennec 2006). Furthermore, viability is probably low among the larvae spawned from histologically healthy ovaries in years of low spat density, because survival was lower in such years than in years of high spat density. These inferences correspond with previous reports, from laboratory experiments, that the nutritional status of broodstock affected the nutritional condition and necrosis of oocytes and the viability of eggs, embryos, and larvae (e.g., Utting and Millican 1998; Barber and Blake 2006).

The effect of low spawner growth on spat density might show a threshold, because the best model (model 2) was not improved when we added adductor muscle weight as an independent variable instead of the categorical variable, which indicates whether a year is a low growth year or not. An adductor muscle weight of 12 g in age-1 scallops in February at Yakumo could be a threshold to judge whether a year is a low growth year or not. Adductor muscle is an important source of stored energy for gametogenesis (Barber and Blake 2006). It was suggested that the high cost of sperm production, involving protein synthesis, cannot be met by stored glycogen reserves, thereby restricting spermatogenesis to periods of high food availability, whereas oogenesis can continue through periods of low food availability (Vahl and Sundet 1985; MacDonald et al. 2006). However, we found no anomaly in the testes in our histological examination.

Besides the environmental data that we considered, other factors might influence spat density, such as winds during the planktonic larval phase and delays in spawning. A strong, seasonal southeasterly wind called "Yamase" usually blows for a few days during the planktonic larval phase (May) in Funka Bay. Wind-advected water masses transport planktonic larvae from Oshima to Iburi, and then the larvae return to Oshima when the wind stops, but densities are lower than before the Yamase in all regions of Funka Bay. The Yamase blows every year, so it might not be a factor in the interannual variation of spat density. In 2005, spawning was delayed probably to the end of May or early June from the usual late April to early May period because of low water temperature. At that time, we observed a large increase in the proportion of necrotic oocytes in Oshima (results not shown). A similar increase in necrotic oocytes due to delayed spawning was reported in *Pecten maximus* (Paulet et al. 1986, 1988). In 2005, the Yamase blew in May as usual, but there were no strong winds during the planktonic phase (June), so the effect of delayed spawning might have been counterbalanced by a reduction of larval drift or loss.

The predicted spat density peaked at $[chl]_{Feb}$ = 5.6 $\mu g \cdot L^{-1}$ and gradually declined as $[chl]_{Feb}$ increased. Higher $[chl]_{Feb}$ levels (>5.6 $\mu g \cdot L^{-1}$) might cause hypertrophy of oocytes. Tada et al. (2006) investigated the relationship between egg diameter and ratio of metamorphosis to D-shaped larva in *M. yessoensis*, under laboratory conditions, by using broodstock sampled weekly at Yakumo in Funka Bay during 2001 to 2004. They found that the metamorphosis ratio gradually increased as egg diameter decreased during the spawning season and egg diameter was largest at the beginning of the 2001 spawning season. These observations may indicate that hypertrophied oocytes are spawned early in the spawning season and their survival may be very low. Their observations are consistent with our interpretation that $[chl]_{Feb}$ above 5.6 $\mu g \cdot L^{-1}$ may cause hypertrophy of oocytes because the highest $[chl]_{Feb}$ was also recorded in 2001.

The correlation between the biomass of spawners and the spat density was not significant. Used as an independent variable, the biomass of the spawners did not improve the models in terms of AIC_c. Therefore, the interannual variations in spat density are not related to spawning biomass. The biomass of the spawners appears to have been always high enough to ensure abundant recruitment when the environment was favorable in Funka Bay.

Concurrence of ENSO and low spat density years and low growth years of spawners

Food availability during gonadal development (February) and whether or not a year was one of low growth of spawners appears to directly determine the spat density. The high concurrences of low $[chl]_{Feb}$ with El Niño and low spawner growth with La Niña suggested that the fundamental environmental factors determining the spat density were El Niño and La Niña. Climatic characteristics of El Niño years might control the $[chl]_{Feb}$ by changing the initiation of spring blooms. Climatic characteristics of La Niña might reduce the growth of scallops in Funka Bay.

Temperate oceans usually have spring and autumn phytoplankton blooms (Evans and Parslow 1985). Spring blooms have been studied for many decades (e.g., Sverdrup 1953; Evans and Parslow 1985), and it was generally accepted that phytoplankton blooms are caused by a combination of vertical stratification of the water column and increasing solar radiation after the replenishment of nutrients by convectional mixing over the winter months. On the other hand, Huisman et al. (1999) explained spring blooms in the absence of vertical stratification (Townsend et al. 1992; Eilertsen 1993). They developed a turbulent diffusion model that distinguishes two mechanisms for the development of phytoplankton blooms: the Sverdrup critical depth model in well-mixed environments, and a critical turbulence threshold by which if turbulent mixing is less than the critical turbulence, phytoplankton growth rates exceed the vertical mixing rates and a bloom develops irrespective of the depth of the upper water layer. Labry et al. (2001) reported winter blooms and inferred that enhancement of light availability, which corre-

sponded with halostratification or decrease of the vertical light attenuation, initiated winter phytoplankton blooms, which are responsible for the exhaustion of phosphate in early spring. Autumn blooms initiate soon after the start of convectional mixing and do not occur under conditions of either very gradual or very rapid deepening of the upper mixed layer (Findlay et al. 2006).

In Funka Bay, durations of winter blooms seemed to affect the initiation of the spring bloom in both high and low spat density years, because spring blooms initiated earlier when winter blooms were shorter. The longer winter bloom might deplete nutrients more severely, so it might take more time to replenish nutrients for the initiation of spring blooms, and spat density might become very low if the spring bloom (i.e., the food availability) did not start until February, the most important time for gonadal development. From AMeDAS climatic data, we sought out the most plausible climatic factors that extended winter blooms in low spat density years. In such years, warmer air temperature and less windy conditions in winter might weaken the vertical mixing of the water column, thus drawing out the winter bloom by extending the period when turbulent mixing is less than the critical turbulence. These are typical characteristics during El Niño years. We also sought out the most plausible climatic factors that influenced spawner growth. In low growth years, convectional mixing might start later and persist longer because of higher water and air temperatures in summer; this delay and longer convectional mixing probably contributed to the low chl *a* concentration from November to January. These are typical characteristics during La Niña years. The warm water temperature in summer and low phytoplankton concentration in winter probably reduced the growth of the scallop. The optimum temperatures for maximum growth of *M. yessoensis* are 4 to 15 °C (Yamamoto 1964). The concurrences of El Niño with low spat density and La Niña with low spawner growth were very high and highly significant. From the results of ecosystem model simulations and single-year field observations in 1983, Azumaya et al. (2001) suggested that the stability of the water column due to warming might play an important role in the onset of the spring bloom in Funka Bay. However, the variability of warming is probably not a factor accounting for interannual variation in the initiation of the spring bloom, because air temperatures were apparently higher during winter–spring and spring blooms initiated later in low spat density years than in high spat density years. From observations of peaks of spring blooms during 1988 to 1992, Kudo and Matsunaga (1999) inferred that the timing of the spring bloom was strongly dependent on the inflow of Oyashio water, which is characterized by relatively lower density, into Funka Bay. This inflow is indicated by a salinity change from 33.6 psu (typical Funka Bay winter water) to less than 33.3 psu (Oyashio water) (Ohtani 1971). The Oyashio inflow did not initiate the spring bloom; our CTD data indicated that initiations of the blooms corresponded with the inflows of Oyashio water in only three years (1997, March; 2000 and 2001, February). The enhancement of light availability, inferred from AMeDAS data collected in Yakumo city, was the most plausible factor behind the blooms' peaks in March. Sunshine had two peaks, in April and August, and greatly increased between February (mean ± standard error (SE): 3.14 ± 0.13 h·day^{-1}) and May (4.18 ± 0.15 h·day^{-1}).

El Niño and La Niña apparently influenced the reproduction and growth of scallops in Funka Bay, possibly by controlling the initiation of winter–spring blooms and summer water temperatures. El Niño and La Niña occurred before 1992, but spat collection failure was first reported in 1992, three years after scallop production reached more than 10^5 t in Funka Bay (Hokkaido Prefectural Government 1982–2007). The influence of ENSO might be strengthened during years of high scallop production because of the large requirement for food during gonadal development.

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Epiphytic macroinvertebrate communities on Eurasian watermilfoil (*Myriophyllum spicatum*) and native milfoils *Myriophyllum sibericum* and *Myriophyllum alterniflorum* in eastern North America

Sarah J. Wilson and Anthony Ricciardi

Abstract: Aquatic macrophytes play an important role in the survival and proliferation of invertebrates in freshwater ecosystems. Epiphytic invertebrate communities may be altered through the replacement of native macrophytes by exotic macrophytes, even when the macrophytes are close relatives and have similar morphology. We sampled an invasive exotic macrophyte, Eurasian watermilfoil (*Myriophyllum spicatum*), and native milfoils *Myriophyllum sibericum* and *Myriophyllum alterniflorum* in four bodies of water in southern Quebec and upstate New York during the summer of 2005. Within each waterbody, we compared the abundance, diversity, and community composition of epiphytic macroinvertebrates on exotic and native *Myriophyllum*. In general, both *M. sibericum* and *M. alterniflorum* had higher invertebrate diversity and higher invertebrate biomass and supported more gastropods than the exotic *M. spicatum*. In late summer, invertebrate density tended to be higher on *M. sibericum* than on *M. spicatum*, but lower on *M. alterniflorum* than on *M. spicatum*. Our results demonstrate that *M. spicatum* supports macroinvertebrate communities that may differ from those on structurally similar native macrophytes, although these differences vary across sites and sampling dates. Thus, the replacement of native milfoils by *M. spicatum* may have indirect effects on aquatic food webs.

Résumé : Les macrophytes aquatiques jouent un rôle important dans la survie et la prolifération des invertébrés dans les écosystèmes d'eau douce. Les communautés d'invertébrés épiphytes peuvent être modifiées par le remplacement des macrophytes indigènes par des marcophytes exotiques, même lorsque ces macrophytes sont de proches parents et possèdent une morphologie similaire. Nous avons échantillonné un macrophyte exotique envahissant, le myriophylle à épis (*Myriophyllum spicatum*), et des myriophylles indigènes (*Myriophyllum sibericum* et *Myriophyllum alterniflorum*) dans des plans d'eau du sud du Québec et du nord de l'état de New York durant l'été 2005. Dans chaque plan d'eau, nous avons comparé l'abondance, la diversité et la composition de la communauté de macroinvertébrés épiphytes sur les *Myriophyllum* exotique et indigènes. En général, tant *M. sibericum* que *M. alterniflorum* portent une plus grande diversité et une biomasse plus importante d'invertébrés ainsi qu'un plus grand nombre de gastéropodes que le *M. spicatum* exotique. En fin d'été, la densité des invertébrés tend à être plus importante sur *M. sibericum* que sur *M. spicatum*, mais plus faible sur *M. alterniflorum* que sur *M. spicatum*. Nos résultats démontrent que *M. spicatum* porte des communautés de macroinvertébrés qui peuvent différer de celles qui se retrouvent sur les macrophytes indigènes de structure similaire, bien que ces différences puissent varier d'un site à un autre et d'une date d'échantillonnage à l'autre. Ainsi, le remplacement des myriophylles indigènes par *M. spicatum* peut avoir des effets indirects sur les réseaux alimentaires aquatiques.

[Traduit par la Rédaction]

Introduction

Aquatic macrophytes play key ecological roles in lakes and rivers, primarily as a source of primary productivity and as a habitat for abundant and diverse faunal communities (Kershner and Lodge 1990; Killgore et al. 1991; Toft et al. 2003). In particular, submersed macrophyte beds support dense communities of invertebrates and thus serve as important feeding areas for fish (Rozas and Odum 1988; Strayer and Malcom 2007). Such communities are being transformed by introductions of invasive exotic macrophytes that dominate or replace native macrophytes (Boylen et al. 1999; Myers and Bazely 2003). Exotic macrophytes can alter the physical and chemical environment, particularly when their growth form and life history traits differ from those of the native species they replace (Carpenter and Lodge 1986; Cattaneo et al. 1998; Caraco and Cole 2002). However, the effects of macrophyte species replacements on aquatic communities remain relatively unexplored (but see Keast 1984; Strayer et al. 2003; Toft et al. 2003). It is

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of interest to know if morphologically similar exotic and native macrophytes will support similar epiphytic macroinvertebrate communities.

Within a given ecosystem, native macrophytes and their associated invertebrates may have interacted over the course of their evolution. If shared evolutionary history matters, then we would expect native macrophytes to support more diverse invertebrate communities than exotic macrophytes in North American inland waters because the vast majority of freshwater invertebrate species in North America are native (Pennak 1989; Merritt and Cummins 1996). Furthermore, exotic macrophytes might facilitate the establishment and population growth of exotic invertebrates, particularly if these species have shared an evolutionary history — a prediction of the "invasional meltdown" hypothesis (Simberloff and Von Holle 1999). Exotic macrophytes might also act as a vector for the introduction of exotic invertebrates (Johnson et al. 2001; Toft et al. 2003). For these reasons, we would expect to find more exotic invertebrates living on exotic macrophytes.

Physical and chemical properties of aquatic macrophytes may influence the abundance, diversity, and community composition of epiphytic invertebrates, potentially resulting in different invertebrate communities associated with different macrophyte species (Cyr and Downing 1988; Chilton 1990; Cheruvelil et al. 2002). Structurally complex macrophytes with finely divided leaves tend to support higher densities of epiphytic invertebrates compared with those with simple leaves (Krull 1970; Cheruvelil et al. 2000, 2002). The chemicals exuded by aquatic macrophytes may also directly affect the abundance of certain invertebrates (Marko et al. 2005), whereas other exudates may influence the growth of epiphytic algae — an important invertebrate food source (Wium-Andersen et al. 1982; Cattaneo 1983; Gross et al. 1996). Therefore, it follows that even macrophytes with similar structure may support contrasting invertebrate communities.

Our objective was to determine if the invasive exotic macrophyte Eurasian watermilfoil (*Myriophyllum spicatum*) supports invertebrate communities equivalent in composition, diversity, and abundance to those on structurally similar native *Myriophyllum* species. *Myriophyllum spicatum* was introduced into North America in the early 1900s, reaching the St. Lawrence River by 1957 (Aiken et al. 1979; Smith and Barko 1990). It is now distributed across North America, where it often outcompetes native milfoils and other aquatic macrophytes through crowding and shading (Boylen et al. 1999). Milfoils are submersed, canopy-forming macrophytes that occur either in mixed or monospecific beds in ponds, lakes, and lentic areas of large rivers (Aiken et al. 1979). Milfoils generally have finely divided, feathery leaves arranged in whorls of four around the stem. Native milfoils *Myriophyllum sibiricum* and *Myriophyllum alterniflorum* and the exotic *Myriophyllum spicatum* are very similar in their growth form and leaf morphology (Gerber and Les 1994; Crow and Hellquist 2000); *M. sibiricum* and *M. spicatum* have similar-sized leaves, whereas *M. alterniflorum* has slightly smaller leaves. If leaf structure (i.e., complex versus simple leaves) largely determines epiphytic invertebrate abundance and diversity, milfoils should support similar invertebrate communities. However, assuming that biochemical or subtle physical differences between these species may provoke adaptations in epiphytic invertebrates (Gross et al. 1996; Marko et al. 2005), we hypothesized that native species of *Myriophyllum* support more abundant and diverse invertebrate communities than *M. spicatum* because of their shared evolutionary history with native invertebrates. Particular attention was given to gastropods because of their important contribution to epiphytic invertebrate biomass. We also hypothesized that *M. spicatum* supports more abundant and diverse exotic invertebrates than native species of *Myriophyllum*. We tested these hypotheses in a multisite field study in which the density, biomass, diversity, and community composition of epiphytic invertebrates on the exotic *M. spicatum* were compared with native *M. sibiricum* and *M. alterniflorum*.

Materials and methods

Study sites

Macrophytes and their associated epiphytic invertebrates were sampled on multiple occasions at four sites in eastern North America: the Richelieu River and Lac St-Louis (a fluvial lake on the St. Lawrence River) in southern Quebec and Upper Saranac Lake and Upper Chateaugay Lake in the Adirondack State Park in upstate New York (Fig. 1). These lakes and rivers represent a range of freshwater habitats in which native and exotic *Myriophyllum* co-occur (Table 1). Prior to sampling, we visually surveyed plants in each waterbody in June or July and selected a site within each where the relative abundance (percent cover) of both native and exotic *Myriophyllum* species was approximately equal. All subsequent samples were then taken from the same site. In Lac St-Louis and the Richelieu River, we sampled *M. spicatum* and the native *M. sibericum*, whereas in Saranac Lake and Chateaugay Lake, we sampled *M. spicatum* and the native *M. alterniflorum*. Two weeks prior to sampling, Saranac Lake was undergoing a milfoil control program wherein *M. spicatum* was manually harvested at discrete periods by divers who took care to leave native macrophytes intact and minimize disturbance in the lake. As a result, the relative abundance of the two species varied throughout the season at this site. At all four waterbodies, we sampled two to three times at monthly intervals in the summer of 2005.

Epiphytic invertebrate sampling

At each site, a snorkeler or SCUBA diver collected macrophytes and epiphytic invertebrates using a modified Downing box sampler, a plexiglas box that encloses plant material and associated invertebrates in 6 L of water (Downing 1986; Rasmussen 1988). On one side of this box is a screen (0.5 mm) to drain water while keeping macroinvertebrates and macrophytes inside. Within each site, we limited the depth variation at which samples were taken to within 1 m. Macrophytes were identified in the field prior to sampling. Because *M. spicatum* and *M. sibericum* are sometimes difficult to distinguish (Crow and Hellquist 2000), we only sampled at sites where morphological differences between the two species were sufficiently pronounced to ensure correct identification. At each site, we collected 6–10 replicate samples of each macrophyte species, except at Saranac Lake where additional samples were taken because

Fig. 1. Map of study sites in southern Quebec and upstate New York, 2005. In Lac St-Louis and the Richelieu River (Quebec), native *Myriophyllum sibiricum* and exotic *M. spicatum* were sampled, and in upper Saranac Lake and upper Chateaugay Lake (New York), native *M. alterniflorum* and exotic *M. spicatum* were sampled.

there were relatively few invertebrates on the macrophytes. The number of samples taken at each site depended on the density of invertebrates and the availability of macrophytes, but sampling effort was equal for both native and exotic macrophytes. Samples were taken from the interior of a mixed bed of macrophytes at sites within 20 m of the shoreline. We made an effort to collect native and exotic macrophytes from similar locations within each site. At each site on each sampling date, we measured water temperature and depth, identified the other macrophyte species present, and took water samples that were transported in a cooler to the lab within 3 h and kept refrigerated (for <48 h) until their pH was measured using a digital meter (AP63 pH meter, Accumet Portable Laboratory, Fisher Scientific, Ottawa, Ontario).

Sample processing

In the field, we placed macrophytes from each sample into plastic trays and removed loose invertebrates from both the tray and sampler using forceps. Invertebrates were immediately preserved in 70% ethanol. The macrophytes from each sample were then stored in separate plastic bags and transported to the lab in a cooler, where they were refrigerated and processed within 48 h of collection. Working under a dissection microscope, we removed all of the remaining invertebrates from the macrophytes using forceps and preserved them in 70% ethanol. Macrophytes were subsequently rinsed of debris (e.g., silt and marl), blotted to remove excess water, dried in an oven at 65 °C for 16 h until a stable weight was reached, and then weighed on an electronic balance (±0.01 g).

We identified preserved invertebrates to the lowest taxonomic level possible under a dissection microscope. Because the mass of individual species and genera was often insignificantly small, we measured the wet weight (±0.001 g) of invertebrates grouped according to their taxonomic order in each sample. The range of biomass was too small to be measured with any more accuracy using dry weights. Although preservation alters the wet weight of invertebrates, all samples were stored in the same conditions (i.e., temperature and light) and weighed within the same week to minimize error due to desiccation. Invertebrate grouped wet weights were measured within <10 s of their removal from ethanol after gentle blotting with tissue paper.

Statistical analysis

Invertebrate density (number of invertebrates per gram of plant dry weight) and biomass (wet weight of invertebrates per gram of plant dry weight) were calculated for each sample and ln-transformed prior to statistical analysis to meet assumptions of normality. Homogeneity of variance across sampling groups at each site was verified using Levene's test. A repeated-measures analysis of variance (ANOVA) was used to compare the mean invertebrate density on native and exotic *Myriophyllum* at each site, with date as the within-subjects factor and plant type (native or exotic) as the between-subjects factor; the same was done for invertebrate biomass. At one site (Richelieu River), a significant interaction effect between sampling date and macrophyte species was found, which prompted us to run post hoc comparisons in each date using Bonferroni-corrected t tests. Densities of exotic invertebrate species on native and exotic *Myriophyllum* were compared using repeated-measures ANOVA when the abundance of an exotic invertebrate species was normally distributed across a site. Otherwise, we used two-tailed t tests to compare the density of an exotic invertebrate species on each sampling date that density was normally distributed — which occurred for all dates except when no exotic invertebrates were found on either native or exotic macrophytes. All statistical tests were preformed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, Illinois).

To measure invertebrate diversity, we calculated taxa richness, the Shannon–Wiener index, and the inverted Simpson index. The latter indices are calculated based on both taxa richness and evenness, but the Shannon–Wiener index is influenced more by taxa richness than by the Simpson index (Magurran 2004). For each site, we created individual-based Coleman rarefaction curves for taxa richness and accumulation curves for Shannon–Wiener and inverse Simpson diversity on native and exotic *Myriophyllum* (Colwell 2005). The curves were generated separately for native and exotic macrophytes at each site and were used to compare invertebrate diversity on two species of macrophytes while controlling for differences in the total abundance of invertebrates on the different macrophyte species. Shannon–Wiener diversity and inverse Simpson diversity were calculated based on 50 randomizations sampled with replacement to generate error bars (Colwell 2005). We also ran a detrended correspondence analysis (DCA) to reveal trends in inverte-

Table 1. Characteristics of sampling sites in 2005.

Site	Location	Plants sampled	System type	No. of samples (E, N)[a]	Water temperature: minimum, maximum (°C)[b]	pH range	Site depth (m)	Other plant species[c]	Notes
Lac St-Louis	Southwest of the Island of Montreal, Quebec; part of the St. Lawrence River	*M. spicatum*, *M. sibericum*	Fluvial lake	7, 6 (June) 6, 6 (July) 6, 7 (August) **19**, **19**	21 (June), 27 (August)	7.9–9.4	0.5–1.0	*C. demersum*, *E. canadensis*, ***P. crispus***, *P. perfoliatus*, *Potamogeton* sp., *Ranuculus* sp., *V. americana*	Site near boat launch against breaker; moderate to high wave action
Richelieu River	Central Quebec, connects Lake Champlain and the St. Lawrence River	*M. spicatum*, *M. sibericum*	River	10, 10 (July) 9, 9 (August) 7, 7 (September) **26**, **26**	19 (September), 24 (July and August)	8.2–9.1	1.5–2.0	*C. demersum*, *E. canadensis*, *E. nuttallii*, ***P. crispus***, *P. richardsonii*, *Potamogeton* sp., *Ranuculus* sp., *V. americana*	Site near midriver, in a wetland; steady current, low wave action
Upper Saranac Lake	Adirondack State Park, upstate New York	*M. spicatum*, *M. alterniflorum*	Lake	21, 21 (July) 18, 18 (August) **39**, **39**	19 (August), 21 (July)	7.7–7.9	1.5–2.0	*C. demersum*, *E. canadensis*, *P. crispus*, *P. richardsonii*, *V. americana*	Site of *M. spicatum* control program; low wave action
Upper Chateaugay Lake	Adirondack State Park, upstate New York	*M. spicatum*, *M. alterniflorum*	Lake	7,7 (July) 9,9 (August) **16**, **16**	17 (August), 21 (July)	6.7–7.8	1.0–1.5	*E. canadensis*, *P. richardsonii*, *Ranuculus* sp., *Utricularia* sp., *V. americana*	Site near lake inflow and wetland, south end of lake; low wave action

[a]Number of samples of exotic (E) and native (N) macrophytes (month sampled is indicated in parentheses); the total number for each is in bold.

[b]Month sampled is indicated in parentheses.

[c]Exotic macrophyte species are in bold. Macrophyte genera: *C*, *Ceratophyllum*; *E*, *Elodea*; *P*, *Potamogeton*; *V*, *Vallisneria*.

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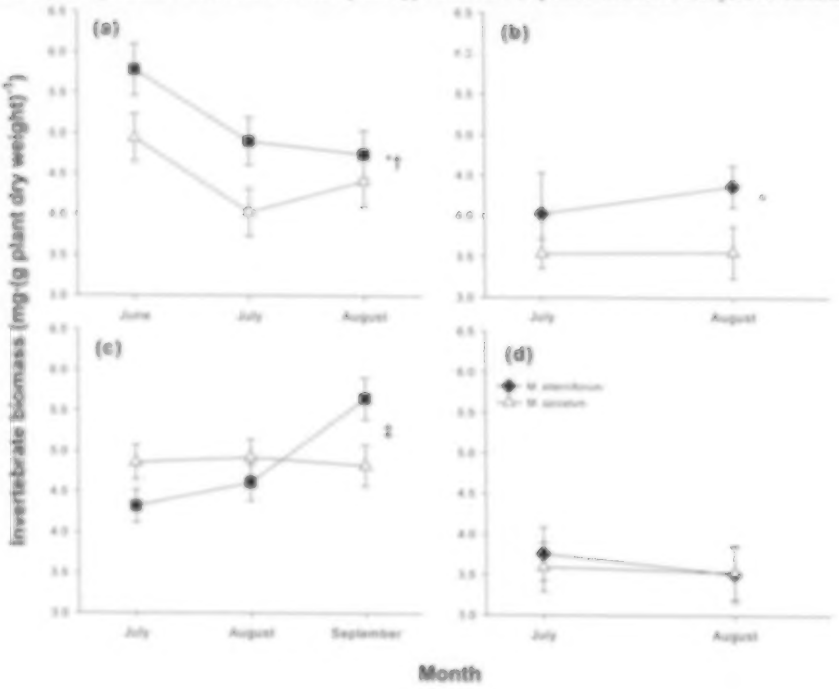
Fig. 2. Invertebrate biomass (milligrams wet weight per gram of plant dry weight) on exotic and native *Myriophyllum* species (±1 standard error, SE) in (*a*) Lac St-Louis, (*b*) Chateaugay Lake, (*c*) Richelieu River, and (*d*) Saranac Lake: solid squares, *M. sibericum*; solid diamonds, *M. alterniflorum*; open triangles, *M. spicatum*; *, a significant difference in invertebrate biomass on native and exotic plants; †, a significant difference between dates; ‡, a significant interaction between plant type and date (repeated-measures analysis of variance, $p < 0.05$).



brate community composition at each site, using CANOCO version 4.5 (Biometris, Wageningen). Before running a DCA, we excluded rare taxa, which were defined as those species present in less than three replicate samples at a site throughout the season.

Results

Invertebrate abundance

At three of the four sites, invertebrate biomass was significantly higher on native *Myriophyllum* species than on *M. spicatum* during at least one sampling period in the summer (ANOVA, $p < 0.05$; Fig. 2). In early to mid-summer, invertebrate biomass was higher on native *M. sibericum* than on *M. spicatum* in Lac St-Louis by a factor of 2.2, whereas from June to August, total invertebrate biomass declined on both macrophyte species. In the Richelieu River, the invertebrate biomass on *M. sibiricum* was slightly lower than on *M. spicatum* in early summer but by late summer was 2.3 times greater than on *M. spicatum*. In Chateaugay Lake, *M. alterniflorum* had twice as much invertebrate biomass than *M. spicatum* during August, whereas at Saranac Lake, both species had similar invertebrate biomass throughout the season. Gastropod biomass, which constituted a substantial portion (>60% at three sites) of the total invertebrate biomass, was also significantly higher on native macrophytes at Lac St-Louis, Chateaugay Lake, and Richelieu River sites (ANOVA, $p < 0.05$). In Saranac Lake, *M. spicatum* and *M. alterniflorum* supported similar gastropod biomass (Fig. 3).

Within each site, invertebrate density tended to differ on native and exotic *Myriophyllum*, especially in mid- to late summer (Fig. 4). Invertebrate density was higher on *M. sibericum* than on *M. spicatum* (ANOVA, $p < 0.05$). Mean invertebrate density on *M. sibericum* was higher than on *M. spicatum* by a factor of 1.5 in Lac St-Louis (August) and by a factor of 2.2 in the Richelieu River (September). Invertebrate density tended to be higher on *M. spicatum* than on *M. alterniflorum*, although only significantly so at Chateaugay Lake (ANOVA, $p < 0.05$). In August, average invertebrate density on *M. alterniflorum* was lower than on *M. spicatum* by a factor of 2.2 in Chateaugay Lake and by a factor of 1.8 in Saranac Lake. The mean body size (estimated from the ratio of biomass to density) of invertebrates on *M. spicatum* was generally lower than on *M. alterniflorum* (Figs. 2 and 4).

Invertebrate diversity and community composition

In general, the two native species of *Myriophyllum* supported either equivalent or greater invertebrate diversity than *M. spicatum*. Invertebrate taxa richness was consistently higher on *M. alterniflorum* than on *M. spicatum* ($p < 0.05$; Fig. 5; Table 2). Invertebrate taxa richness was also higher on *M. sibericum* than on *M. spicatum* in the Richelieu River ($p < 0.05$), whereas in Lac St-Louis, both macrophytes species supported similar taxa richness. Shannon–

Fig. 3. Gastropod biomass (milligrams wet weight per gram of plant dry weight) on exotic and native *Myriophyllum* species (±1 standard error, SE) in (*a*) Lac St-Louis, (*b*) Chateaugay Lake, (*c*) Richelieu River, and (*d*) Saranac Lake: solid squares, *M. sibericum*; solid diamonds, *M. alterniflorum*; open triangles, *M. spicatum*; *, a significant difference in gastropod biomass on native and exotic plants; †, a significant interaction between plant type and date (repeated-measures analysis of variance, $p < 0.05$).

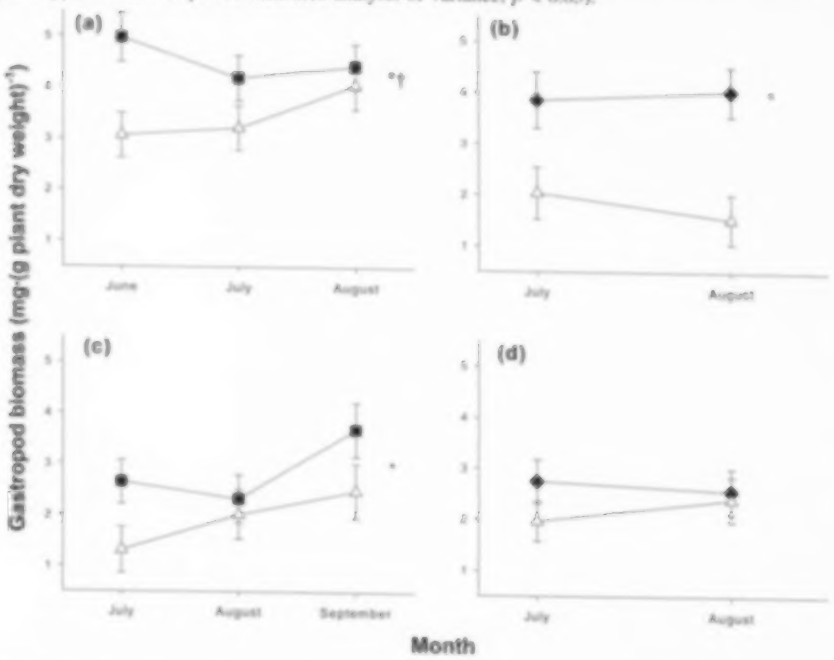


Fig. 4. Invertebrate density (number of invertebrates per gram of plant dry weight) on exotic and native *Myriophyllum* species (±1 standard error, SE) in (*a*) Lac St-Louis, (*b*) Chateaugay Lake, (*c*) Richelieu River, and (*d*) Saranac Lake: solid squares, *M. sibericum*; solid diamonds, *M. alterniflorum*; open triangles, *M. spicatum*; *, a significant difference in invertebrate density on native and exotic plants; †, a significant difference between dates (repeated-measures analysis of variance, $p < 0.05$).

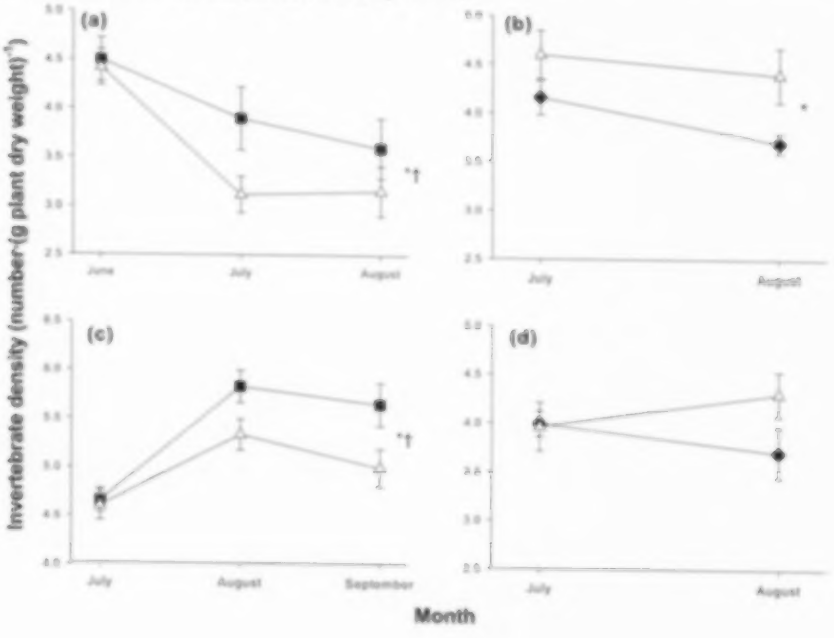


Fig. 5. Rarefaction curves for invertebrate taxa richness on exotic and native *Myriophyllum* species in (*a*) Lac St-Louis, (*b*) Chateaugay Lake, (*c*) Richelieu River, and (*d*) Saranac Lake: solid squares, *M. sibiricum*; solid diamonds, *M. alterniflorum*; open triangles, *M. spicatum*; *, a significant difference in invertebrate taxa richness between the two plant species ($p < 0.05$). Error bars represent standard deviation based on 50 randomized runs.

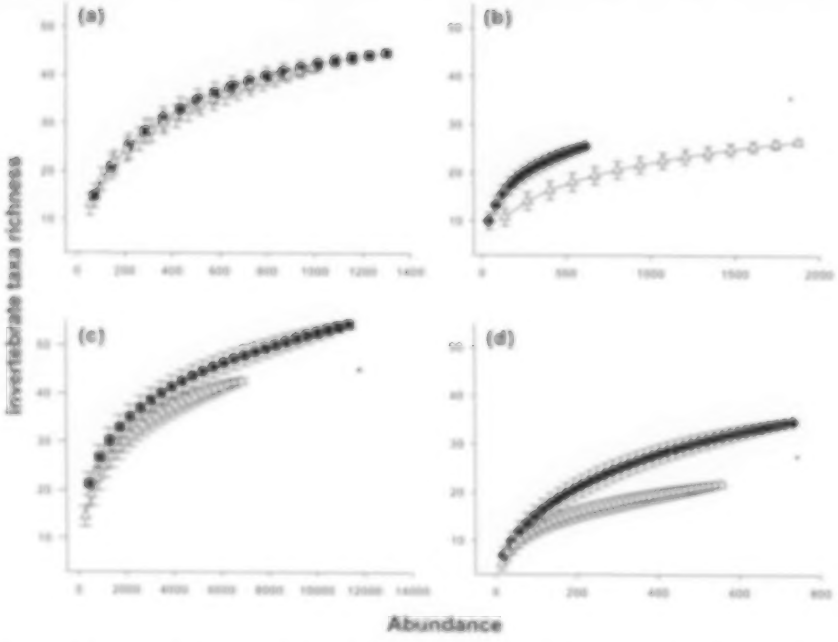


Table 2. Taxa richness, the exponent of Shannon–Wiener diversity ($e^{H'}$), and the inverse Simpson diversity ($1/D'$) compared on native and exotic *Myriophyllum* species.

	Taxa richness		$e^{H'}$		$1/D'$		
Site	Native	Exotic	Native	Exotic	Native	Exotic	Invertebrate abundance[a]
Lac St-Louis	40.4	42	7.5	7.8	4.2	4.3	1050
Richelieu River	45.4	43*	4.6	3.6*	2.8	2.1*	7020
Saranac Lake	31.5	22*	6.5	4.8*	3.6	2.8	580
Chateaugay Lake	26	18*	8.3	4.3*	6.1	3.1*	650

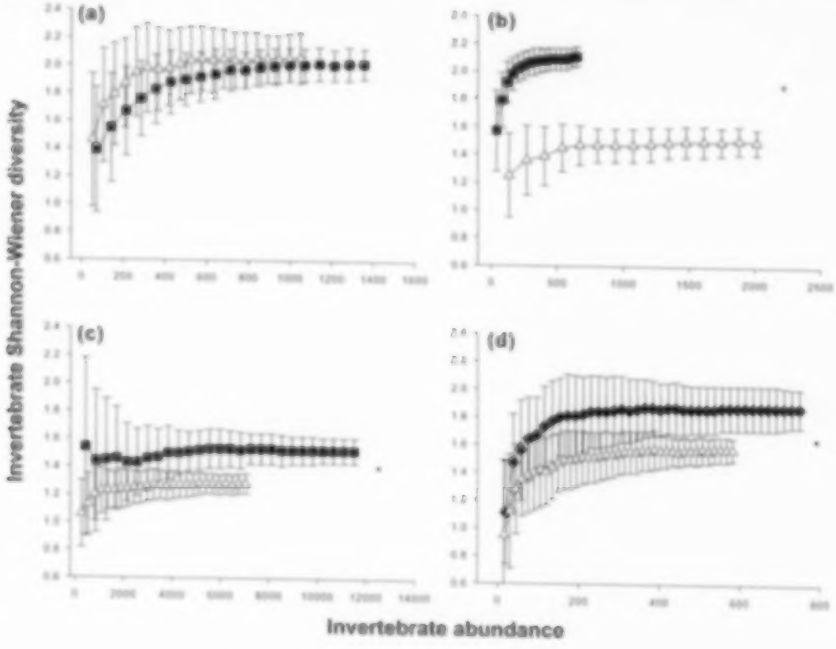
Note: Both the exponent of Shannon-Wiener diversity and the inverse Simpson diversity indices represent the number of different species that we would find if all species in the community were equally common (Jost 2006); however, the inverse Simpson index is more sensitive to how evenly invertebrate abundance is distributed across taxa. Significantly different values ($p < 0.05$) are indicated by an asterisk (*).

[a]Diversity comparisons on native and exotic macrophytes were made at the level of abundance of invertebrates on the macrophyte species with the fewest invertebrates.

Wiener diversity followed the same pattern as taxa richness (Fig. 6; Table 2). The inverse Simpson index showed a similar pattern, with the exception of Saranac Lake; at this site, *M. alterniflorum* tended to have higher inverse Simpson diversity of invertebrates than *M. spicatum*, but the difference was not significant (Table 2). Thus, across all sites, taxa richness, Shannon–Wiener diversity, and the inverse Simpson diversity index of invertebrates on native milfoils were equal to or significantly higher than on *M. spicatum* (Table 2).

Although native and exotic *Myriophyllum* supported many of the same invertebrate species, the abundance of certain invertebrate groups (e.g., gastropods) was often different on each milfoil species. At three of our four sites, both species of native *Myriophyllum* had higher gastropod biomass per gram of plant dry weight than *M. spicatum* (ANOVA, $p < 0.05$). The differences between invertebrate community composition on *M. alterniflorum* and *M. spicatum* were particularly distinct in Chateaugay Lake. At this site, detrended correspondence analysis showed that samples divided along two axes: the first representing plant type, and the second representing sampling date (Figs. 7*a*, 7*b*). Generally, *M. spicatum* supported many chironomids and oligochaetes, whereas *M. alterniflorum* supported many amphipods and gastropods. Invertebrate communities associated with *M. spicatum* were characterized by an abundance of naidid oligochaetes (Ni), chironomids (Ch), caddisflies of the hydroptilid genus *Oxyethira* (Hx), and the Eurasian pyralid moth, *Acentria ephemerella* (= *Acentria nivea*) (Ac) (Fig. 7*b*). Invertebrates characteristic of *M. alterniflorum* were the gastropods *Valvata sincera* (Vs) and *Amnicola limosa* (Am), the amphipod *Hyallela azteca* (Hy), damselflies in the fam-

Fig. 6. Shannon–Wiener diversity of invertebrates on exotic and native *Myriophyllum* species in (*a*) Lac St-Louis, (*b*) Chateaugay Lake, (*c*) Richelieu River, and (*d*) Saranac Lake: solid squares, *M. sibericum*; solid diamonds, *M. alterniflorum*; open triangles, *M. spicatum*; *, a significant difference in invertebrate Shannon–Wiener diversity between the two plant species ($p < 0.05$). Error bars represent standard deviation based on 50 randomized runs.



ily Coenagrionidae (Co), and caddisflies of the genus *Orthotricha* (Ho). Invertebrate communities were also different in early and late summer, with more planarians on both macrophyte species in July and more caddisflies in August (Fig. 7*b*). In Saranac Lake, differences in invertebrate communities were not clearly defined by either date or macrophyte species.

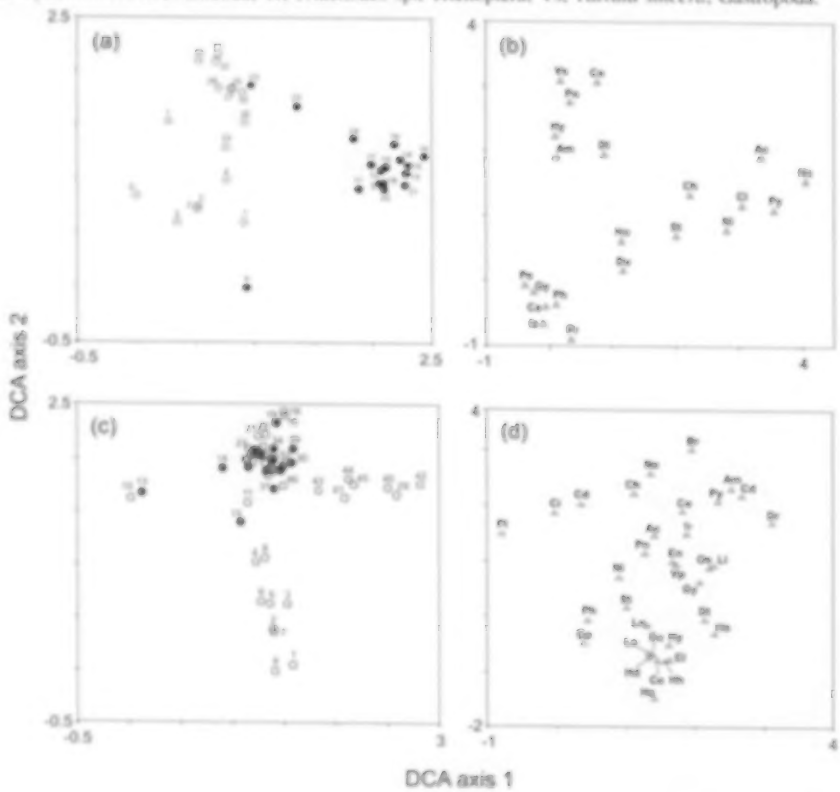
Invertebrate community composition also differed between *M. spicatum* and *M. sibericum* in the Richelieu River (Figs. 7*c*, 7*d*), while such differences were less apparent in Lac St-Louis. In the Richelieu River, as in Chateaugay Lake, *M. spicatum* supported many chironomids and oligochaetes, whereas *M. sibericum* supported many amphipods and snails. Invertebrate communities characteristic of *M. spicatum* were dominated by chironomids (Ch), oligochaetes (Ni, St), and the caddisfly *Brachycentrus* sp. (Br) throughout the sampling period. Invertebrate communities on *M. sibericum* were characterized by the amphipod *Hyalella azteca* (Hy), the gastropods *Physa gyrina gyrina* (Ph), *Gyraulus circumstriatus* (Gy), and *Amnicola limosa* (Am), the planarian *Dugesia tigrina* (Du), ostracods (Os), and in September, the Eurasian zebra mussel, *Dreissena polymorpha* (Dr). Generally, the invertebrate composition on *M. spicatum* did not vary throughout the season, whereas those on *M. sibericum* changed in each sampling period: in July, *M. sibericum* had many gastropods and amphipods; in August, invertebrates were similar to those on *M. spicatum*, with many naidid oligochaetes (Ni), chironomids (Ch), and *Brachycentrus* sp. (Br); in September, *M. sibericum* supported many zebra mussels (Figs. 7*c*, 7*d*). In Lac St-Louis, gastropods were significantly higher on *M. sibericum* than on *M. spicatum* (ANOVA, $p < 0.05$), but otherwise invertebrate communities were not clearly differentiated by either date or macrophyte species.

Exotic invertebrates

Three species of exotic invertebrates were found. The Eurasian moth *Acentria ephemerella* was present at all four sites and was higher on *M. spicatum* than on the native *Myriophyllum* species at every site. In Saranac Lake, we found only two specimens of *A. ephemerella* in one sample of *M. spicatum* and none on *M. alterniflorum*. In Chateaugay Lake, *A. ephemerella* again was not found on *M. alterniflorum* but was present in 38% of the samples of *M. spicatum* and thus was grouped with *M. spicatum* in the DCA analysis (Figs. 7*a*, 7*b*). In the Richelieu River and Lac St-Louis, *A. ephemerella* occurred on both *M. sibericum* and *M. spicatum* but was more likely to group with the Eurasian milfoil in the Richelieu River (Figs. 7*c*, 7*d*). In the Richelieu River, *A. ephemerella* was present in 38% of the *M. spicatum* samples and 27% of the *M. sibericum* samples, and in Lac St-Louis, these frequencies were 16% and 10%, respectively.

In Lac St-Louis, the abundance of the Eurasian snail *Bithynia tentaculata* did not differ significantly between *M. sibericum* and *M. spicatum* on any of the sampling dates (Fig. 8*a*). In the Richelieu River, the density of *Dreissena polymorpha* was significantly higher on *M. sibericum* than on *M. spicatum* in September after the peak period of zebra mussel settlement (*t* test, $p < 0.01$; Fig. 8*b*).

Fig. 7. Detrended correspondence analysis of the invertebrate communities at (*a*, *b*) Chateaugay Lake and (*c*, *d*) the Richelieu River. At Chateaugay Lake (*a*), numbers up to 16 are from July, those greater than 16 are from August; in the Richelieu River (*c*), numbers from 1 to 21 are from July, 22 to 40 are from August, and 41 to 55 are from September. Open circles in (*a*) and (*c*) represent *Myriophyllum spicatum*. Solid circles in (*a*) and (*c*) represent the native species *M. alterniflorum* and *M. sibiricum*, respectively. Species codes (*b*, *d*) are as follows: Ac, *Acentria ephemerella* (*nivea*), Lepidoptera; Am, *Amnicola limosa*, Gastropoda; Br, *Brachycentrus* sp., Trichoptera; Ca, *Caenis* sp., Ephemeroptera; Cd, Cladocera; Ce, Ceratopogonidae, Diptera; Ch, Chironomidae, Diptera; Ci, *Chimarra* sp., Trichoptera; Cl, Cladocera; Co, Coenagrionidae, Odonata; Dr, *Dreissena polymorpha*, Mollusca; Dt, *Dugesia tigrina*, Turbellaria; du, *Dugesia tigrina*, Turbellaria; El, Elmidae, Coleoptera; En, *Enallagma* sp., Odonata; Eu, *Eubrychiopsis lecontei*, Coleoptera; Ga, *Gammarus* sp., Amphipoda; Gy, *Gyraulus circumstriatus*, Gastropoda; Hd, Hydracarina; He, *Helobdella* sp., Annelida; Ho, *Orthotrichia* sp., Trichoptera; Hx, *Oxyethira* sp., Trichoptera; Hy, *Hyalella azteca*, Amphipoda; Is, Isopoda; Le, Leptoceridae, Trichoptera; Li, *Leptocerus* sp., Trichoptera; Nc, *Nectopsyche* sp., Trichoptera; Ne, *Nehalennia* sp., Odonata; Ni, Naididae, Annelida; Os, Ostracoda; pe, *Promenetus exacuous exacuous*, Gastropoda; Ph, *Physa gyrina gyrina*, Gastropoda; Pl, *Platycentropus*, Trichoptera; Po, *Polycentropus* sp., Trichoptera; Pp, *Pristina* sp., Annelida; Py, Pyralidae, Lepidoptera; st, *Stylaria lacustris*, Annelida; Tr, *Triaenodes* sp., Trichoptera; Vs, *Valvata sincera*, Gastropoda.



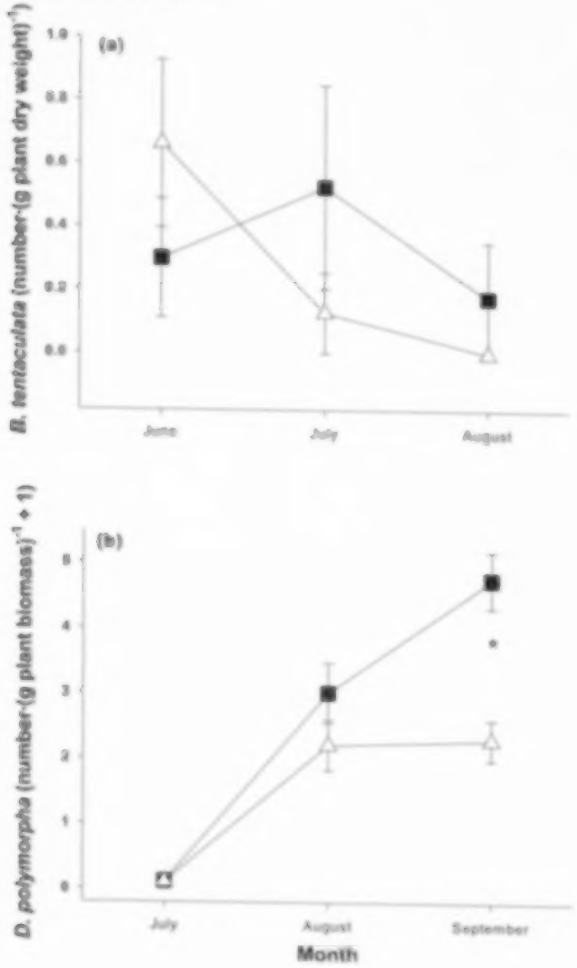
Discussion

Invertebrate abundance and body size

Our results show that invertebrate community structure can differ substantially on exotic and native milfoils of similar morphology. However, the relationship between invertebrate abundance on these macrophytes varies throughout the summer, with differences in invertebrate density becoming more pronounced later in the season. Given that *M. spicatum* grows rapidly in the spring (Grace and Wetzel 1978), it may be the first macrophyte species to provide substrate and resources to colonizing invertebrates. Indeed, at our sampling sites, *M. spicatum* grew earlier than both species of native *Myriophyllum*, but contrary to the expectation that *M. spicatum* would support more invertebrates early in the summer, there was generally no difference in invertebrate density at that time. Invertebrates may require a few months to differentially colonize macrophyte species, and it may be that subtle differences among macrophytes (e.g., involving chemical exudates or epiphytic algal communities; Balci and Kennedy 2003; Marko et al. 2005) have significant effects on invertebrate abundance only by late summer.

Different species of aquatic macrophytes support epiphytic algal communities that may differ greatly in abundance and taxonomic composition (Blindow 1987; Cattaneo et al. 1998; Laugaste and Reunanen 2005). Algae growing on macrophytes in the St. Lawrence River and surrounding areas reach peak biomass by mid-June or July (Cattaneo 1983; Gosselain et al. 2005), and so we would expect differences in the epiphytic algal communities of native and exotic *Myriophyllum* to be more apparent in midsummer. Contrasting abundances or composition of epiphytic algae

Fig. 8. Density of exotic molluscs: (*a*) the faucet snail *Bithynia tentaculata* in Lac St-Louis (±1 standard error, SE), and (*b*) the zebra mussel *Dreissena polymorpha* in the Richelieu River (±1 SE): solid squares, *M. sibiricum*; open triangles, *M. spicatum*; *, a significant difference in invertebrate abundance on native and exotic plants (*t* test, $p < 0.01$).



on macrophyte species could drive dissimilarities in epiphytic invertebrate communities that are structured around algae as a food source; for example, Cattaneo (1983) found that the abundance of invertebrate grazers increased substantially a short time after peak epiphyte biomass.

Our study examined gastropod biomass in particular, because gastropods graze on epiphytic algae and consume macrophytes directly (Lodge 1986; Sheldon 1987; Brönmark 1989) and also they comprised a substantial proportion of invertebrate biomass in our samples. They had significantly higher biomass on native *Myriophyllum* species at three of the four sites. *Myriophyllum sibiricum* and *M. spicatum* have very similar structure and approximately equal plant surface area per gram of dry plant weight (Gerber and Les 1994), so differences in gastropod biomass between the two species cannot be attributed to the area available for colonization by gastropods or epiphytic algae. Given that gastropod abundance may vary with epiphytic algal biomass and composition (Lodge 1986; Brönmark 1989), differences in the epiphytes themselves on native and exotic milfoils may be driving this result. Whatever the mechanism, gastropods have been found to preferentially inhabit certain macrophyte species over others, even when these macrophytes are closely related (Pip and Stewart 1976; Sheldon 1987).

The mean body size of invertebrates was also lower on *M. spicatum* than on *M. alterniflorum*, as indicated by an equal or higher invertebrate biomass combined with a lower invertebrate density on *M. alterniflorum*. This result may reflect differences in invertebrate community composition, as *M. alterniflorum* supported larger snails and amphipods, whereas *M. spicatum* was dominated by smaller annelids and chironomids.

Macrophyte structure

Submersed aquatic macrophytes are often grouped into two main structural categories based on leaf morphology: dissected (complex) leaves or entire (simple) leaves (Krecker 1939; Cheruvelil et al. 2002). By these standards, the three species of *Myriophyllum* in our study are similar in structure as all have dissected leaves arranged in whorls of four around the stem. On a finer scale, however, there are structural differences among species. *Myriophyllum alterniflorum* tends to branch more frequently and has slightly smaller leaves than either *M. spicatum* or *M. sibiricum*. *Myriophyllum alterniflorum* also has significantly higher specific leaf area (surface area of leaf per dry weight of leaf) and surface area to volume ratios than the other species, whereas *M. spicatum* and *M. sibiricum* have very similar specific leaf area and leaf surface area to volume ratios (Gerber and Les 1994). In addition, dry leaf mass comprises ~55% of the total dry weight of leaves and shoots of *M. alterniflorum* and ~65% for both *M. spicatum* and *M. sibiricum* (Gerber and Les 1994). Hence, subtle differences in leaf morphology exist between *M. alterniflorum* and *M. spicatum*, but *M. spicatum* and *M. sibiricum* have quite similar leaf morphology. The range of whole plant surface area – biomass ratios on *M. spicatum* and *M. sibiricum* also overlap, varying between 320 and 1205 $cm^2 \cdot g^{-1}$ on *M. spicatum* (Kowalczewski 1975; Sher-Kaul et al. 1995) and between 534 and 761 $cm^2 \cdot g^{-1}$ on *M. sibiricum* (Gerrish and Bristow 1979; Armstrong et al. 2003). Thus, although subtle structural differences may account for the dissimilarity in invertebrate communities on *M. alterniflorum* and *M. spicatum*, they are less likely to be responsible for differences in invertebrates on *M. sibiricum* and *M. spicatum*.

It seems probable that characteristics other than plant structure influence invertebrate abundance and diversity on these macrophytes. Chemical exudates may affect the relative palatability of exotic and native milfoils to herbivores and the growth of epiphytic algae. Marko et al. (2005) found that the milfoil weevil, a specialist herbivore native to North America, prefers *M. spicatum* to its native host *M. sibiricum* because of differences in the amount of glycerol and uracil exuded by the two species. Previous studies have also found that *M. spicatum* releases chemicals that deter the growth of certain types of epiphytes and that these chemicals may dif-

Table 3. Studies examining comparing invertebrates on *Myriophyllum spicatum* to those on native macrophytes in invaded regions.

Study	Region	Sampling year	Native plants	Invertebrate	
				Taxa richness	Density
Keast 1984	Southeast Canada	1979	*Potamogeton robbinsii*, *Vallisneria americana*	N/A	E > N (47, 25)
Chilton 1990	Midwest US	1983	***Ceratophyllum demersum***	N/A	E < N (253, 676)
Cheruvelil et al. 2000	Midwest US	1998	***Ranunculus*** sp., *Potamogeton pectinatus*	N/A	E < N (75, 77)
Krull 1970	Northeast US	1966	***Ceratophyllum demersum***, ***Elodea canadensis***, ***Utricularia vulgaris***, *Potamogeton pectinatus*, *Lemna trisulca*, *Najas flexilis*, *N. marina*, *Heteranthera dubia*, *Rhizoclonium hieroglyphicum*	E < N (18, 33)	E < N (36, 100)
Balci and Kennedy 2003	Southwest US	1998	*Heteranthera dubia*	E < N (21, 24)	E > N[a] (2276, 1888)
Krecker 1939	Northeast US	1935–1936	***Elodea canadensis***, *Potamogeton pectinatus*, *P. compressus*	E > N (22, 14)	E > N[b] (1442, 535)

Notes: E, exotic macrophyte (*M. spicatum*); N, native macrophyte(s). Plants with similar structure (divided leaves) to *M. spicatum* are indicated in bold. Density is the number of invertebrates per unit plant weight, unless otherwise indicated. N/A, not available.

[a]Number of invertebrates per m^2 of plant surface area.

[b]Number of invertebrates per 10 m length of plant stem.

fer in either the type or the amount exuded relative to other species of *Myriophyllum* (Gross et al. 1996).

Exotic invertebrates

The invasional meltdown hypothesis postulates that previously established exotic species facilitate the invasion or proliferation of other exotic species (Simberloff and Von Holle 1999) and thus predicts that *M. spicatum* would support more exotic invertebrates than either *M. sibericum* or *M. alterniflorum*. However, this prediction is not supported by the results of our study. The abundance of the Eurasian snail *Bithynia tentaculata* was not significantly different on native and exotic macrophytes. *Bithynia tentaculata* established in the Great Lakes and St. Lawrence River basin more than a century ago, several decades prior to the invasion of *M. spicatum* (Aiken et al. 1979; Mills et al. 1993), and therefore may have had sufficient time to adapt to native macrophytes. In the Richelieu River, the native *M. sibericum* supported more zebra mussels than *M. spicatum*. It seems unlikely that differences in plant exudates or epiphytic algae would have caused this result, as zebra mussels are filter feeders with larvae that are planktonic and eventually settle on solid surfaces, including submersed macrophytes. Given that larval dispersal is largely determined by vagaries of water currents (Lewandowski 1982), it is likely that small-scale variation in hydrology within a site determines the degree to which the mussels colonize macrophytes.

The Eurasian moth *A. ephemerella* was the only exotic invertebrate that appeared to preferentially colonize *M. spicatum*. The density of *A. ephemerella* was slightly higher on *M. spicatum* than on either native species of *Myriophyllum* at all four sites, and DCA analysis showed that this moth tended to group with *M. spicatum* in the Richelieu River and Chateaugay Lake. Not a single *A. ephemerella* was collected from samples of *M. alterniflorum* in either Chateaugay Lake or Saranac Lake, whereas at both of these sites, *A. ephemerella* was found on *M. spicatum*, perhaps indicating that *A. ephemerella* prefers to colonize the exotic milfoil. This moth was introduced from Europe and was first discovered in the St. Lawrence River in 1927 (Berg 1942; Sheppard 1945). It is a generalist herbivore that feeds on many species of macrophytes (Buckingham and Ross 1981) but has been considered as a possible biological control agent for *M. spicatum* as it may cause less damage to native macrophytes (Johnson et al. 1997).

Implications for aquatic communities

To our knowledge, this is the first study to compare multispecies invertebrate assemblages on native and exotic species of *Myriophyllum*. Previous studies that examined differences between epiphytic invertebrates on *M. spicatum* and native macrophytes found conflicting results (Table 3), perhaps because each compared *M. spicatum* with macrophytes of differing phylogeny, structure, and life history traits.

The establishment of *M. spicatum* can reduce the density of many other species of macrophytes, including native milfoils (Grace and Wetzel 1978; Smith and Barko 1990; Boylen et al. 1999). In situations where *M. spicatum* supports lower invertebrate diversity and biomass than its congeners, the displacement of native macrophytes by *M. spicatum* could conceivably cause habitat-wide alterations in epiphytic invertebrate communities that have consequences for higher trophic levels. Reductions in the diversity, biomass, and mean body size of epiphytic invertebrates may impact fish and other vertebrate predators by lowering the quality and accessibility of prey (Werner and Hall 1974; Eggers 1977; Kovecses et al. 2005). Overall, these results suggest that the impacts of *M. spicatum* invasion on epiphytic invertebrates may have multiple indirect effects at local and habitat-wide scales. Structurally similar, even congeneric, macrophytes do not necessarily provide equivalent habitat for invertebrate communities, which appear to be sensitive to subtle differences between macrophyte species.

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Movements of mottled sculpins (*Cottus bairdii*) in a Michigan stream: how restricted are they?

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Abstract: We monitored 94 mottled sculpins (*Cottus bairdii*) marked with passive integrated transponder (PIT) tags to examine seasonal movement patterns. High detection efficiency (mean ± standard error = 0.76 ± 0.03) of PIT-tagged fish was obtained with a portable antenna. Nighttime searches were more effective than daytime relocations in summer, and smaller fish (<65 mm) were detected more readily than larger fish (≥65 mm). Sculpins used erosional habitats more than depositional habitats, particularly during summer and nighttime. Search attempts within a 150 m reach showed that displacement, mean movement distance, and home range did not differ significantly between summer and winter, suggesting that season did not strongly influence movement. Additionally, movement metrics were unrelated to sculpin size. However, supplementary searches of a larger reach (700 m) showed that 16% of PIT-tagged fish moved more than 100 m over 1 year, with a maximum displacement of 511 m, and mobile fish typically displayed upstream bias. Although most mottled sculpins in our system were relatively sedentary, a small proportion of individuals moved further than previously reported for other systems.

Résumé : Nous avons suivi 94 chabots tachetés (*Cottus bairdii*) porteurs de transpondeurs intégrés passifs (PIT) afin d'étudier leurs patrons de déplacements saisonniers. Nous avons obtenu une efficacité élevée de détection (moyenne ± écart type = 0,76 ± 0,03) des poissons marqués de PIT au moyen d'une antenne portative. Les recherches de nuit sont plus efficaces que les relocalisations de jour en été et les poissons plus petits (<65 mm) sont plus facilement décelables que les poissons plus grands (≥65 mm). Les chabots utilisent les habitats d'érosion plus que les habitats de sédimentation, particulièrement en été et durant la nuit. Des tentatives de recherche sur une section de 150 m ont montré que la distance moyenne des déplacements et l'aire vitale ne diffèrent pas significativement durant l'été et l'hiver, ce qui laisse penser que la saison n'influence pas fortement les déplacements. De plus, les métriques des déplacements sont sans relation avec la taille des chabots. Cependant, des recherches additionnelles sur une section plus grande (700 m) ont montré que 16 % des poissons munis de PIT se déplacent de >100 m sur une année avec un déplacement maximal de 511 m et les poissons mobiles montrent typiquement une préférence pour l'amont. Bien que la plupart des chabots tachetés dans notre système soient relativement sédentaires, un petit pourcentage d'individus se déplace sur des distances plus grandes qu'il n'a été observé dans d'autres systèmes.

[Traduit par la Rédaction]

Introduction

Information on the movements of stream fishes provides valuable insight into a variety of population processes in streams (Skalski and Gilliam 2000; Rashleigh and Grossman 2005; Roberts and Angermeier 2007*a*). A long-held theory characterizing the movements of stream fishes, termed the restricted movement paradigm, is that resident adult fish are sedentary and often spend their lives in short (<50 m) stream reaches (Gowan et al. 1994; Rodriguez 2002). However, movement studies that were used to formulate, question (i.e., are stream fish movements really restricted?), and modify (i.e., populations are heterogeneous with sedentary and mobile components) the restricted movement paradigm typically focused on salmonids (Gowan et al. 1994; Rodriguez 2002), even though small-bodied, non-game species are dominant components of many streams (Grossman et al. 1998). Studies examining the mobility of small-bodied benthic fishes are sparse (relative to salmonids) but necessary to infer general patterns and causes of movements by stream fishes (Petty and Grossman 2004).

Freshwater sculpins (*Cottus* spp.) dominate fish assemblages in many small streams in terms of both abundance and biomass (e.g., Freeman et al. 1988) and are widely distributed across the northern hemisphere (Scott and Crossman 1998). Sculpins can comprise an important forage base for large-bodied fishes in some systems (Dineen 1951; Moyle 1977) and can affect benthic macroinvertebrate communities

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(Kohler and McPeek 1989; Dahl and Greenberg 1996) by benthic foraging (Bailey 1952; Gerking 1994). Sculpin movements are often thought to be highly restricted. For instance, median movement distances were 1.3 m over 45 days in an Appalachian stream (Petty and Grossman 2004). However, studies examining diel and seasonal movement patterns of sculpins are relatively rare (e.g., Knaepkens et al. 2004, 2005; Keeler 2006).

Common techniques used to assess stream fish movement generally do not accommodate small-bodied fishes. For instance, because of tag size, most radiotelemetry devices are limited to marking larger fish (Brown et al. 1999, 2006). Alternatively, fin clips and pigment marks can be used to individually mark small-bodied fishes (Nielsen 1992), but subsequent recapture (e.g., electrofishing) or visual relocation (e.g., snorkeling) of marked fish is necessary to assess movement. Passive integrated transponder (PIT) technology has recently gained popularity (e.g., Cunjak et al. 2005; Quintella et al. 2005; Bubb et al. 2006) because PIT tags are long-lasting and tagged individuals can be relocated without being recaptured (Nielsen 1992), minimizing bias associated with common mark–recapture protocols (Gibbons and Andrews 2004).

We monitored movements of PIT-tagged mottled sculpins (*Cottus bairdii*) in a small headwater stream. Our objectives were (*i*) to estimate detection probabilities of PIT-tagged mottled sculpins to evaluate the efficacy of using a portable underwater PIT antenna for estimating their movements, (*ii*) to examine mottled sculpin movement patterns by estimating displacement and home range, and (*iii*) to estimate temporal variability in detection efficiency and movement estimates by monitoring mottled sculpins on a diel and seasonal basis.

Materials and methods

Study site

Seven Mile Creek is a second-order stream in Calhoun County, Michigan. Hourly water temperatures (mean ± 1 standard error (SE); Optic StowAway® Temp; Onset Computer Corp., Bourne, Mass.) were 18.67 ± 0.21 °C during summer (July–August 1999–2005) and 2.08 ± 0.32 °C during winter (December–January 1998–2005). Flow is highly stable because of extensive groundwater inputs and this stream drains a forested wetland with relatively little development (Kohler and Wiley 1997). Our study site was a 150 m stream reach consisting of both erosional (i.e., high velocity, gravel and cobble substrata) and depositional (i.e., low velocity, sand and silt substrata) habitats. The 150 m section was comprised of a 50 m core reach with 50 m upstream and downstream buffer zones (Fig. 1). Stream morphology is not characterized by typical riffle–pool sequences (e.g., Allan 1995); rather, Seven Mile Creek is dominated by erosional habitats (70%) with areas of slower moving water along stream margins (i.e., depositional habitats, 30%). Mean wetted width is 5.76 ± 0.23 m, and erosional and depositional habitats support benthic macroinvertebrate assemblages unique to each zone (Breen 2008). Backpack electrofishing surveys conducted in September 2006 suggested that mottled sculpins dominated the fish assemblage. We estimated (based on the Lincoln–Peterson method with Chapman correction; Pollock et al. 1990) mottled sculpin densities to be 114 individuals per 100 m^2 within our 50 m core study reach (see below). American brook lamprey (*Lampetra appendix*), blackside darter (*Percina maculata*), brown trout (*Salmo trutta*), central mudminnow (*Umbra limi*), creek chub (*Semotilus atromaculatus*), western blacknose dace (*Rhinichthys obtusus*), johnny darter (*Etheostoma nigrum*), rainbow darter (*Etheostoma caeruleum*), and white sucker (*Catostomus commersoni*) were present in much smaller numbers.

Coordinate system designation and equipment calibration

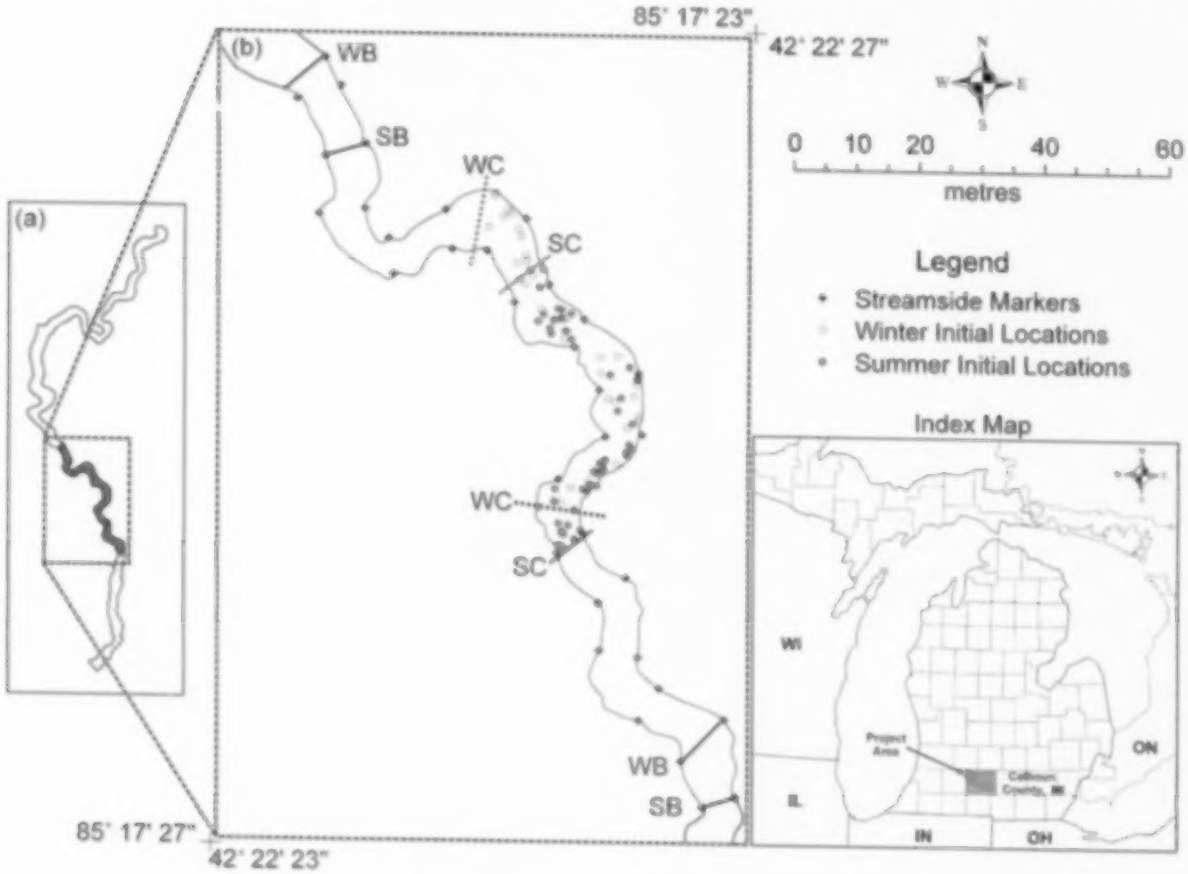
Permanent markers were established along both stream banks of the 150 m study site to determine the locations of PIT-tagged fish. Stream banks were marked with survey stakes at ~10 m intervals (i.e., paired reference points). Individual global positioning system (GPS) files were collected at each stake (GPS Pathfinder Pro XRS 12-channel differential receiver; Trimble Navigation Limited, Sunnyvale, Calif.). Real-time differential corrections were applied during the field survey, and individual GPS files were postprocessed (Pathfinder Office; Trimble Navigation Limited) to gain additional accuracy (±5–10 cm). Corrected GPS files were exported as individual geographic information system (GIS) files using ArcView® 3.3 shape file format (Environmental Systems Research Institute, Inc., Redlands, Calif.).

Once permanent markers were in place, we conducted a calibration exercise to determine the probability of detecting PIT tags (Bubb et al. 2002). A colleague hid 50 PIT tags on the stream bottom throughout the core reach so that they were not readily visible to the operator (e.g., Linnansaari et al. 2007). The study site was searched using a multidirectional antenna (30.5 cm Triangle waterproof antenna) mounted on a 3 m telescopic pole connected to an antenna tuning box and portable reader (FS2001F-ISO; Biomark, Inc., Boise, Idaho). While walking upstream in the wetted channel, the operator scanned the contour of the stream bottom from one bank to the other (Roussel et al. 2000). The antenna was submerged in areas of deeper water (>15 cm) to stay within tag detection limits (17–30 cm; Cucherousset et al. 2005). Water depth, habitat type (erosional or depositional), and distance from PIT tags to numbered weights (see Sampling protocol) were recorded where each tag was relocated.

Sampling protocol

Over the course of our study, 94 mottled sculpins (about 25% of total population) were captured via three-pass removal backpack electrofishing in the core reach (Fig. 1*b*), marked with PIT tags, and released. Two size classes, small adults (total length (TL) < 65 mm, mean = 59.4 ± 0.6 mm TL, N = 29) and large adults (TL ≥ 65 mm, mean = 73.4 ± 0.9 mm TL, N = 65), were examined to determine intrapopulation variation in movement (range = 52–100 mm TL). Fifty mottled sculpins (17 small adults and 33 large adults) were collected and tagged during the summer of 2006 and 44 (12 small adults and 32 large adults) were collected and tagged during the winter of 2006–2007. Fish were anesthetized with tricaine methanesulfonate (MS-222; 50 mg·L^{-1}), measured (TL), and injected with a 12.45 × 2.02 mm PIT tag (134.2 kHz; TX1411SST; Biomark, Inc.) using a 12-

Fig. 1. (*a*) Section of Seven Mile Creek (Calhoun Co., Michigan) that was searched for PIT-tagged mottled sculpins (*Cottus bairdii*; 5 July 2006 to 29 June 2007). The 700 m site (upstream boundary, 42°37.654′N, 85°28.947′W; downstream boundary, 42°37.037′N, 85°29.093′W) includes a designated study reach (shaded region) and extended areas that were searched to locate fish moving outside the study reach (unshaded). (*b*) Enlargement showing permanent streamside markers (~10 m apart along both stream banks) that separated a 50 m core study section (SC, summer core reach; WC, winter core reach), where all fish were initially tagged and released, from 50 m upstream and downstream buffer zones (SB, summer buffers; WB, winter buffers), which were searched to account for fish that moved from initial tagging sites.



gauge syringe-style implanter following the protocol described by Ruetz et al. (2006). Once fish were tagged, individuals were held in buckets located in the stream for a minimum of 2 h to verify survival and tag retention before fish were released at the location of capture. We observed no mortalities during initial tagging and only three fish (range = 51–54 mm TL) did not retain their tags when first marked (these individuals were not included in the number of marked fish). Similarly, evaluations of the efficacy of implanting PIT tags in small-bodied fishes showed high retention rates (Keeler 2006; Ruetz et al. 2006; Knaepkens et al. 2007) with negligible adverse effects on foraging and swimming behavior (Knaepkens et al. 2007; Newby et al. 2007) or growth and survival (Ruetz et al. 2006; Knaepkens et al. 2007; Cookingham and Ruetz 2008).

PIT-tagged mottled sculpins were monitored within the 150 m study site to determine detection probabilities and movement patterns. Fish were relocated every two weeks during daytime (0900–1500) and nighttime (1900–0200) searches for three consecutive days during the summer (6 July – 17 August 2006) and 1.5 consecutive days (two daytime and one nighttime searches) during the winter (9 December 2006 – 21 January 2007) with a portable underwater antenna. Fish were initially tagged on 5 July 2006 during the summer and on 29 November 2006 during the winter. The duration of summer and winter tracking was 43 and 53 days, respectively. Fish relocations were made in an upstream direction (mean duration = 2.6 ± 0.1 h), beginning at the lower boundary of the study section (Fig. 1*b*), using the same scanning protocol described for the calibration exercise. Keeler et al. (2007) reported that search direction (i.e., upstream or downstream) did not bias movement results, and searching in the downstream direction was not logistically practical at our site because of limited visibility. A 700 m stream section (excluding the 150 m reach; Fig. 1*a*) was also searched in conjunction with summer and

winter tracking to account for fish that moved outside of the main study reach. Extended searches (mean duration = 6.4 ± 0.3 h), which required two days to complete on two occasions, were conducted for summer fish on 9 November 2006 – 29 June 2007 (N = 10, duration = 359 days) and for winter fish on 17 December 2006 – 29 June 2007 (N = 7, duration = 212 days). Additionally, fish observations obtained within the 150 m study reach during population estimates (N = two searches), winter fish collections (N = one search), and a mottled sculpin diet study (N = five searches; Breen 2008) were used to calculate maximum displacement (see Statistical analyses and movement estimation).

When a PIT-tagged fish was located, we used a numbered weight to mark their exact position, passed the antenna over the location a second time to determine if the fish maintained its location, and noted habitat type (erosional or depositional). To evaluate if mottled sculpins were startled during this procedure (i.e., relocation procedure affected behavior; Hill et al. 2006), we made a determination of whether fish moved when detected by an operator (Cookingham and Ruetz 2008). We determined whether fish were startled for all relocations during both seasons. To minimize disturbance, only one operator was in the stream scanning for fish while another person recorded observations from the stream bank.

For each fish's location, we measured the distance and azimuth (handheld compass) of the numbered weight to an adjacent streamside marker. This information was entered into GIS software to obtain x and y coordinates for each location. Variation in measurements from streamside markers to numbered weights was minimal (5.5 ± 1.3 cm, n = 15 replicate measurements made on 29 March 2007). Once a daytime search of the 150 m study site was complete, we measured all fish locations. However, fish locations for nighttime searches were measured the following day.

Over a 1-year period, a total of 52 PIT tags (summer, N = 37; winter, N = 15) were recovered from the stream bottom, having been lost from live or dead individuals. To avoid biasing movement data, we carefully assessed observations originating from shed tags. We considered three criteria to validate previous observations: (*i*) upstream movement, (*ii*) lateral movement > 1 m, or (*iii*) fish were startled during a relocation attempt. If at least one of these conditions was met, we included the observation in our analyses. The first observation for a given individual that did not meet any of the criteria was considered the time of death (or tag loss). We excluded the time of death and all observations thereafter (e.g., Keeler et al. 2007) to avoid underestimating movement. During the winter period, we verified whether tagged individuals that remained at the same location during an entire relocation period (1.5 consecutive days) were alive by gently moving nearby substrate.

Tag loss in our study was most likely due to natural mortality because mottled sculpins are short-lived (≤age 4 in Wisconsin streams; Becker 1983), and we tagged older fish (individuals >65 mm TL are usually age 3). We discount the likelihood that many live mottled sculpins lost their tags because tag retention was high (96%–100%) over 1–4 months in laboratory studies (Keeler 2006; Ruetz et al. 2006), and PIT tags were retained for a mean duration of 37.5 ± 7.4 days among fish that lost tags, which is adequate time for insertion wounds to heal in mottled sculpins (Ruetz et al. 2006). Additionally, we discount the likelihood that mortalities were caused by tagging because mortalities associated with tagging fish tend to occur soon (1–2 days) after tag insertion (Cunjak et al. 2005; Ruetz et al. 2006). Finally, terrestrial (e.g., birds and mammals) and aquatic (e.g., brown trout) predation was probably not a large source of mortality for PIT-tagged mottled sculpins in Seven Mile Creek given their cryptic nature (Keeler et al. 2007), that we retrieved tags from the stream bottom, and the rare occurrence of brown trout in Seven Mile Creek.

Statistical analyses and movement estimation

Detection probabilities, calculated for individual fish (i.e., each fish was an experimental unit), were compared across size classes, diel cycle, and seasons. To make diel and seasonal comparisons, detection probabilities were calculated for relocations within the 150 m study reach because we did not begin extended searches until 9 November 2006. We used the Shapiro–Wilk test statistic (α = 0.05) to determine the normality of each data set (including the use of standard transformations) and whether standard parametric or nonparametric statistics were most appropriate. Additionally, we used a χ^2 test for equal proportions to determine whether mottled sculpins displayed specific habitat selection strategies for erosional, depositional, or both (i.e., consistently switching between them) habitat types. Fish were classified as erosional ($P_E > 0.67$), depositional ($P_E < 0.33$), or switching between habitat types ($0.33 \leq P_E \leq 0.67$), where P_E was the proportion of observations for an individual fish in erosional habitat.

Mottled sculpin movement was indexed by spatial displacement during subsequent relocations using several methods. Fish locations were analyzed using the ArcView Animal Movement extension version 2.04 (Hooge and Eichenlaub 1997), with individual fish as the experimental unit. First, displacement was calculated as the maximum linear distance observed when considering all relocations of each fish. For some fish, displacement was estimated as the distance following the center of the stream channel (Daugherty and Sutton 2005) to account for meanders (Fig. 1), which would underestimate total distance traveled by individual fish. Second, mean movement distance was estimated by measuring the distance between each consecutive location for each fish. Third, home range was estimated using the minimum convex polygon (MCP) method (White and Garrott 1990). To avoid overestimating home range, we subtracted terrestrial area from MCP estimates (e.g., home range estimates of fish inhabiting a meandering reach may include riparian areas). Directional movement was assessed by assigning positive or negative values to the movements (based on the 10 m section of the study reach in which a fish was located) of each fish and evaluating median distances (Skalski and Gilliam 2000). A χ^2 test was used to compare the number of fish that moved upstream with the number that moved downstream during each season (Skalski and Gilliam 2000). Fish that were not relocated on at least one occasion for displacement and directional movement estimates (four fish) and two occasions for home range and mean movement distance estimates (seven fish) were excluded from analyses. To maintain consistent sampling ef-

fort between seasons, only the first day–night–day sequence from the summer period was analyzed and all formal tests used only movement observations in the 150 m study reach. However, maximum displacement distances for each fish were determined using all movement observations that occurred inside the study reach and extended search areas. A Pearson's correlation coefficient (r_p) was calculated to test for a linear association between initial TL and log-transformed movement metrics (i.e., displacement, mean movement distance, and home range) using all movement observations (inside and outside the study reach).

Results

Efficacy of PIT equipment

We detected 94% of PIT tags during the calibration study, and the accuracy of locating PIT tags was high (mean distance between numbered weights and tag-only treatments was 14.4 ± 1.5 cm). Including all sources of error (GIS corrections, stake measurements, and numbered weight placement), maximum measurement error was estimated to be <30 cm in our study. Mottled sculpins were relocated 11.9 ± 1.3 (summer fish) and 9.2 ± 0.6 times (winter fish) when considering all search attempts, whereas they were relocated 5.2 ± 0.5 and 8.2 ± 0.6 times, respectively, when using 1.5 days of summer data. Of 967 mottled sculpin relocations made with the PIT antenna throughout both study periods, 22% of observations resulted in a fish being startled by the antenna operator (i.e., moved from where they were initially found during a search attempt). Movement by fish immediately (i.e., during same search period) after being startled was minor (1.4 ± 0.12 m, N = 87), suggesting that our procedure did not strongly affect mottled sculpin behavior.

Detection probabilities

Thirty-six search attempts within the 150 m study reach provided 864 locations on 92 mottled sculpins (one fish was never found and one fish was found outside the study reach) during summer and winter. Overall detection probabilities (seasons pooled) were 0.76 ± 0.03. We relocated 99% of PIT-tagged mottled sculpins one or more times, 96% three or more times, 85% five or more times, and 55% on 10 or more occasions. At the conclusion of summer tracking (i.e., last sample period), 55% of the 20 remaining fish were relocated within the 150 m reach (56% of those not found were relocated at a later date). Following winter tracking, 72% of the remaining 39 fish were relocated in the 150 m reach (55% of those not found were relocated at a later date). Detection probabilities calculated for daytime (summer median = 0.83; winter median = 0.88; Mann–Whitney, U = 846.5, P = 0.098, N = 92) and nighttime (summer median = 0.92; winter median = 1.00; Mann–Whitney, U = 998, P = 0.637, N = 92) searches did not differ significantly between seasons. However, nighttime searches were significantly more effective than daytime searches in summer (Sign test, P = 0.001, N = 92) but not winter (Sign test, P = 0.541, N = 92). Detection probabilities were negatively correlated with initial TL (Spearman correlation, r_S = –0.207, P = 0.047, N = 92 fish). Small adults were detected more readily (0.81 ± 0.05) than large adults (0.74 ± 0.04) when seasons were pooled. Although detection probabilities were zero for three fish, two of these individuals were located outside of the study reach during extended searches and were included in movement–size correlations and maximum displacement estimates.

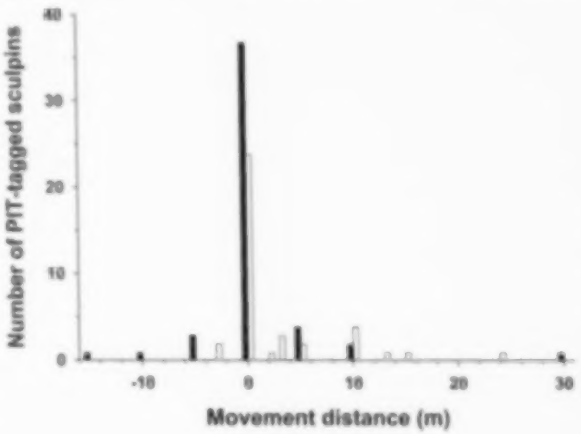


Fig. 2. Median displacement of passive integrated transponder (PIT) tagged mottled sculpins (*Cottus bairdii*) in summer (5 July – 17 August 2006; N = 49 fish; solid bars) and winter (29 November 2006 to 21 January 2007; N = 41 fish; open bars). The first relocation of each fish (day or night) during a seasonal sample period (N = 4 for both seasons) was used to estimate movement distances. Positive and negative distances were assigned based on fish movement from one sample period to the next (i.e., over 2 weeks) following the center of 10 m sections throughout a 150 m reach of Seven Mile Creek.

The probability that a mottled sculpin was detected in an erosional habitat was 0.81 ± 0.03 during summer and 0.61 ± 0.05 in winter, but this did not differ significantly between day (summer, 0.78 ± 0.04; winter, 0.60 ± 0.05) and night (summer, 0.83 ± 0.03; winter, 0.63 ± 0.06) for summer (t = –0.711, P = 0.481, df = 48, N = 49) or winter (Sign test, P = 0.571, N = 40). However, when pooled across seasons, 70% of tagged fish were consistently associated with erosional habitats, 11% were consistently associated with depositional habitats, and 19% routinely switched between habitat types at night (χ^2 = 53.685, P < 0.001, df = 2, N = 89 fish). Similarly, 64%, 14%, and 22%, respectively, utilized these habitats during the daytime (χ^2 = 38.854, P < 0.001, df = 2, N = 89 fish).

Mottled sculpin movement

We conducted 54 search attempts from 5 July 2006 to 29 June 2007, which includes relocation efforts within the 150 m reach (N = 36 searches) and additional searches (N = 18 searches). We obtained 622 movement observations on 90 fish (49 summer and 41 winter fish) when including only the first 1.5 days of summer searches and 1003 movement observations on 93 fish (50 summer and 43 winter fish) when considering all searches during summer and winter. Mottled sculpin movement was not significantly correlated with initial TL when movement was estimated as displacement (r_p = 0.136, P = 0.194, N = 93), mean movement dis-

Table 1. Movements of passive integrated transponder (PIT) tagged mottled sculpins (*Cottus bairdii*) observed in a 700 m section of Seven Mile Creek.

	Summer		Winter	
	Mean (SE)	Median	Mean (SE)	Median
Displacement (m)[a]	62.29 (15.01)	26.01	45.13 (8.72)	16.05
Mean movement distance (m)[b]	6.67 (1.06)	4.33	8.03 (1.89)	3.11
MCP home range (m^2)[c]	123.11 (35.13)	38.50	101.84 (41.93)	15.64

Note: Summer fish were tagged on 5 July 2006 and tracked until 29 June 2007 (N = 50 fish). Winter fish were tagged on 29 November 2007 and tracked until 29 June 2007 (N = 43 fish). SE, standard error.

[a]Displacement is the greatest distance between any two sculpin locations.

[b]Mean movement distance is the distance between each consecutive sculpin location.

[c]Sculpin home range is estimated with the minimum convex polygon (MCP) approach using all locations for each fish.

tance (r_p = 0.080, P = 0.454, N = 91), or home range (r_p = 0.038, P = 0.720, N = 91).

The majority of the mottled sculpins in our study were relatively stationary (Fig. 2) even though some fish moved large distances during both seasons (Table 1; Fig. 3). Displacement (median, summer = 7.97 m, winter = 8.76 m), mean movement distance (median, summer = 2.95 m, winter = 1.91 m), and home range (median, summer = 10.48 m^2, winter = 7.59 m^2) did not differ significantly between seasons (Fig. 3). However, 15 PIT-tagged fish displayed maximum displacements of >100 m when all movements were included (Fig. 4). Estimates of mean and median movements were larger for both seasons when incorporating observations from extended searches outside the 150 m study reach (Table 1). Additionally, we found evidence of upstream bias in movements. Although mottled sculpins showed no significant bias in directional movement during summer (χ^2 = 0.333, P = 0.564, df = 1, N = 12 fish), we found significant upstream bias during winter (χ^2 = 8.067, P = 0.005, df = 1, N = 15 fish; Fig. 2) and all fish with maximum displacements of >100 m moved in the upstream direction.

Discussion

Mottled sculpin movement

We detected higher mobility of mottled sculpins in our study than previously reported for other systems irrespective of season. The maximum detected distance that a mottled sculpin moved was 511 m in <207 days (fish was tagged during summer and the estimate of time scale is conservative because extended searches were not conducted until 9 November 2006). This appears to be the farthest reported displacement for this species even though a perched road culvert blocked further upstream movement at our study site. Furthermore, three fish moved >300 m over a period of 144–207 days, which exceeds the maximum reported displacement of 270 m over 7–8 months for another freshwater cottid, the bullhead (*Cottus gobio*; Knaepkens et al. 2005), and the maximum reported displacement for sculpins (*Cottus* spp.) in a Montana stream (209 m over 25 days; Schmetterling and Adams 2004). Maximum movements of mottled sculpins in the Coweeta drainage (North Carolina) were 54 m over 458 days (Hill and Grossman 1987) and <250 m over a period of about 150 days (Petty and Grossman 2004). Although we detected long-distance movements by mottled sculpins, most individuals were relatively sedentary, which was similar to bullheads in a Flemish river (monitored for up to eight months) that consisted of relatively stationary (i.e., remained in initial sections of capture) and mobile (i.e., moved 10–270 m) individuals (Knaepkens et al. 2004, 2005). This heterogeneity (i.e., stationary and mobile components of the population) likely represents different movement processes that correspond to the leptokurtic distribution of movement distances (Skalski and Gilliam 2000; Fraser et al. 2001; Roberts et al. 2007). Similarly, studies on Potomac sculpin (*Cottus girardi*; Hudy and Shiflet 2009) and Roanoke logperch (*Percina rex*; Roberts et al. 2007), other small-bodied benthic fishes with poor swimming ability, found that most individuals were relatively sedentary (≤30 m for Potomac sculpin over 30–363 days and ≤75 m for Roanoke logperch over 41–720 days) with a small proportion of individuals moving long distances (maximum displacement was 1711 m over 75 days for Potomac sculpin and 3200 m over 800 days for Roanoke logperch).

Mechanisms causing some individuals to move larger distances in Seven Mile Creek are unclear, but we suspect that it may be related to habitat heterogeneity (e.g., Albanese et al. 2004) or sculpin densities and habitat availability (e.g., Grossman et al. 2006). We found that all mottled sculpins that moved distances >100 m (i.e., located outside of our 150 m study reach during extended searches) were in the upstream direction. Decreased habitat complexity (i.e., minimal amounts of cobble, large woody debris (LWD), or deep water) immediately upstream of our study site possibly contributed to large upstream movements because fish going upstream had to move large distances to find suitable habitats. Additionally, we did not detect movement >5 m below the downstream buffer zone, where the stream widens, gradient decreases, and depth and cover decrease directly below the study reach. The region of low habitat complexity extends much further downstream than upstream (M.J. Breen, personal observation), which may explain the lack of long-distance movements downstream. We suspect that reach-scale (e.g., Thompson et al. 2001) differences in the distribution of suitable habitats in Seven Mile Creek leads to higher habitat heterogeneity than in the Coweeta drainage, which may explain differences in sculpin mobility between systems. Alternatively, habitat fragmentation in terms of oc-

Fig. 3. (*a*) Displacement (farthest distance between any two locations for all subsequent relocations of each fish) of passive integrated transponder (PIT) tagged mottled sculpins (*Cottus bairdii*) during summer (5 July – 17 August 2006) and winter (29 November 2006 to 21 January 2007). (*b*) Mean movement distance between consecutive locations for all locations of each fish during the same period. (*c*) Minimum convex polygon (MCP) home range estimates for each fish during the same period. Error bars represent ±1 standard error.

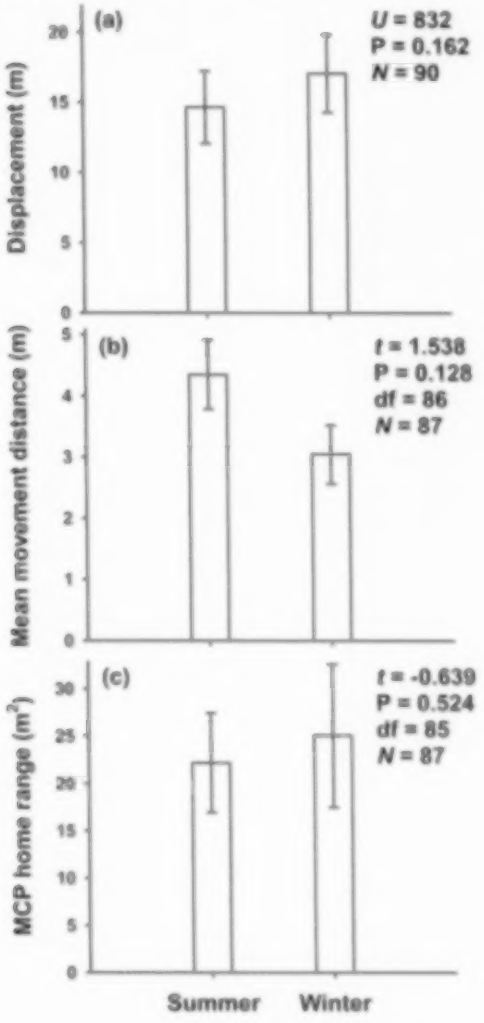
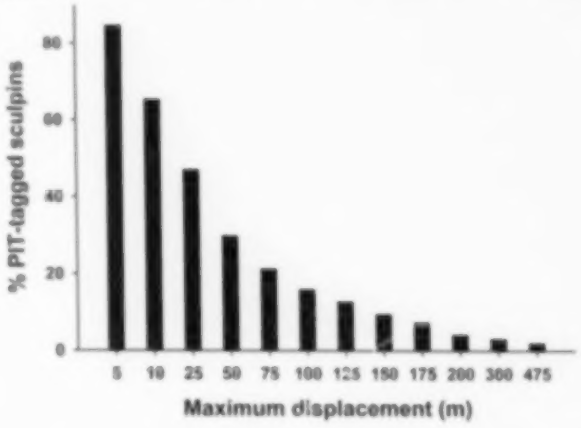


Fig. 4. Percentage (%) of passive integrated transponder (PIT) tagged mottled sculpins (N = 93 fish) with maximum displacements greater than 5 m from 5 July 2006 to 29 June 2007 in Seven Mile Creek.

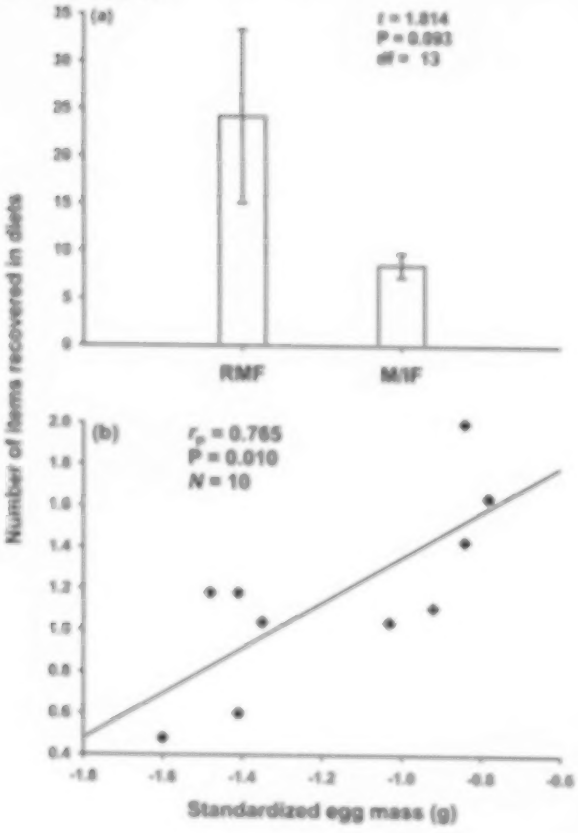


cupancy (i.e., sculpin densities) led to increased movement and settlement of vacant habitats (i.e., unsaturated) by Potomac sculpins (Hudy and Shiflet 2009). We do not have sculpin population estimates for the regions of low habitat complexity in Seven Mile Creek; however, Hudy and Shiflet (2009) found that long-distance movements occurred through habitats with both low and high sculpin densities. Therefore, we hypothesize that benthic fishes will move larger distances in streams where preferred habitat characteristics are patchily distributed (i.e., higher habitat heterogeneity) at the reach scale than in systems where habitat is dispersed homogenously. Nevertheless, patches of low habitat complexity at the reach scale may also impede the movements of some benthic fishes (Roberts and Angermeier 2007*b*).

Our findings, along with the recent work by Hudy and Shiflet (2009), show that sculpins can move hundreds of metres over relatively short (75–200 days) time scales, which has important implications for assessing the movements of small-bodied stream fishes. Past research was often biased against detecting long-distance movements because of a failure to monitor movements over a large area (Gowan et al. 1994; Albanese et al. 2003). In our study, movement metrics increased substantially when we included observations outside of our 150 m study reach. Similarly, Smithson and Johnston (1999) found that several small-bodied stream species were consistently detected outside of initial locations, suggesting that exploratory movements (i.e., outside of normal home ranges) are commonplace. Therefore, incorporating study reaches substantially larger than the maximum reported displacement for a species is likely necessary to detect the most mobile fish in a population. Additionally, we caution against solely evaluating median movement distances, which is common in many studies of stream fishes, because individual fish can move large distances on average but have a median near zero if upstream and downstream movements are offsetting and can underestimate overall movement if movements are continuous in one direction.

Mottled sculpin movements did not differ markedly between summer and winter. To our knowledge, we report the first winter movement estimates for mottled sculpins. However, other lotic species such as brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Oncorhynchus clarkii*; Lindstrom and Hubert 2004), as well as smallmouth bass (*Micropterus dolomieu*; Todd and Rabeni 1989), showed decreased winter activity. Although we expected that mottled sculpin activity would decrease in winter as a result of lower metabolic demand (Wootton 1998), mottled sculpins remained active during both seasons, even though summer water tempera-

Fig. 5. (*a*) Diet analysis of mottled sculpins (*Cottus bairdi*) collected from Seven Mile Creek on 26 January 2007. Stomach contents were collected using gastric lavage, and reproductive status (reproductively mature females (RMF; $N = 10$) and males or immature females (M/IF; $N = 10$)) was determined via dissection. Mean body mass did not differ significantly ($U = 28$, $P = 0.96$) between RMF (3.46 ± 0.80 g) and M/IF (2.43 ± 0.99 g) sculpins. (*b*) Number of prey items present in stomach contents of reproductively mature female mottled sculpins. Egg mass was determined by removing and weighing the entire egg sack of each individual and was standardized by body mass (egg mass / fish mass). Number of prey items and standardized egg mass were log-transformed for statistical analyses.



tures in Seven Mile Creek are about 17 °C warmer than in the winter. Keeler (2006) also detected elevated winter activity for slimy sculpins (*Cottus cognatus*) and proposed that reproductive ecology may play a role. Ovarian recrudescence of mottled sculpins can occur several months before the onset of spawning (Dehaven et al. 1992). Although we did not account for gender when tagging mottled sculpins, dissection of 20 fish opportunistically collected on 26 January 2007 showed that half were gravid females. Reproductively mature females consumed more prey than nongravid females and males (although this difference was not significant), and the number of prey in female diets was positively associated with their standardized egg mass (Fig. 5). Therefore, we suspect that increased winter movements resulted from females actively foraging to increase breeding season fecundity (e.g., Wootton 1998; Roberts et al. 2006).

Seasonal shifts in home range (i.e., consistent use of different areas) were observed for 6 of 10 fish that were monitored during both seasons. Similar observations have been reported for some large-bodied species, such as northern hog suckers (*Hypentelium nigricans*; Matheney and Rabeni 1995) and flathead catfish (*Pylodictis olivaris*; Daugherty and Sutton 2005). This phenomenon may be linked to a host of biotic and abiotic processes, including overwintering protection (Cunjak 1996; Lindstrom and Hubert 2004), prey availability (Petty and Grossman 1996; Thompson et al. 2001), reproductive ecology (Downhower and Brown 1979; Grossman et al. 2002), life-history changes in habitat requirements (e.g., Roberts and Angermeier 2007*a*), and natural disturbance (Natsumeda 2003; Albanese et al. 2004).

Movement patterns of mottled sculpins were not dependent on body size in our study. Likewise, Keeler (2006) did not find a relationship between movement and size of slimy sculpins in Canadian streams. In contrast, juvenile mottled sculpins were more mobile than adults in an Appalachian stream (Petty and Grossman 2004). The discrepancy in size-specific sculpin movement could be system-specific, resulting from density-dependent interactions (Petty and Grossman 2004; Rashleigh and Grossman 2005; Grossman et al. 2006), or a function of the approach. We were limited to marking individuals >55 mm TL with PIT tags (Ruetz et al. 2006). If we could have marked age-0 and juvenile sculpins, then we may have been able to detect a relationship between sculpin size and movement similar to that reported by Petty and Grossman (2004).

Detection probabilities

Portable antennas appear to be a viable technique for assessing movement patterns of PIT-tagged sculpins (and potentially other small-bodied fishes) in small streams. Our detection probabilities of PIT-tagged mottled sculpins were consistently high among temporal scales (day vs. night across seasons) and size class. Our detection probabilities also approached those of other studies on PIT-tagged slimy sculpins in enclosed (0.82; Cucherousset et al. 2005) and open (0.80; Keeler et al. 2007) stream sections. Nevertheless, a limitation of this portable PIT equipment is a maximum detection distance of 17–30 cm (Cucherousset et al. 2005), which can make searching complex habitats difficult (Hill et al. 2006), limits use to shallow areas (e.g., Cucherousset et al. 2007), and may cause some fishes to flee due to the close proximity of the antenna (Cookingham and Ruetz 2008). Additionally, searching extended areas with portable underwater antennas is labor intensive. However, failure to detect mobile fish that move outside a study reach will underestimate fish movement (Gowan et al. 1994; Gowan and Fausch 1996). Alternatively, stationary antennas can be placed across small stream channels (e.g., Bond et al. 2007) on upstream and downstream borders of a study section, allowing researchers to identify individuals that move outside of a reach.

We identified temporal differences in detection probabilities of PIT-tagged mottled sculpins at the diel scale but not at the seasonal scale. Winter detection probabilities of mottled sculpins were not higher than in summer as we ex-

pected, probably because winter sculpin activity was similar to summer. However, mottled sculpins were detected by the underwater antenna more easily at night, especially during summer. We suspect that diurnal feeding behavior of mottled sculpins (Kohler and McPeek 1989) may play a role because sculpins may utilize LWD more during the day, thus making them more difficult to detect (Hill et al. 2006). We found fish in erosional habitats more often at night, suggesting that they were more likely to be in open areas (i.e., exposed) at nighttime and hidden underneath complex cover (i.e., LWD) during daytime. Similarly, Japanese fluvial sculpins (*Cottus pollux*) were observed on open sandy substrates more often at nighttime than during daytime (Natsumeda 1998), Lake Michigan mottled sculpins were found on exposed silt beds more often at nighttime (Hoekstra and Janssen 1985), and banded sculpins (*Cottus carolinae*) were found on tops of rocks at nighttime and beneath rocks during daytime (Greenberg and Holtzman 1987).

Detection probabilities were also dependent on sculpin size. Smaller individuals were detected more readily than larger fish, although this relationship was weak. We hypothesize that large PIT-tagged mottled sculpins were associated with LWD habitats more often than smaller fish, thus making them more difficult to detect. Habitat patches with LWD may be more desirable because of higher macroinvertebrate densities (Breen 2008) and larger mottled sculpins are likely more successful at acquiring preferred patches (e.g., Petty and Grossman 1996, 2004, 2007).

In conclusion, mottled sculpins were relatively sedentary, with 16% of individuals moving distances >100 m over the course of our study. In contrast to much of the literature on seasonal movements of stream fishes, our study showed that mottled sculpins were active during both summer and winter, which we suspect is related to reproductive ecology of this species. Finally, our findings that sculpin movements can be substantial highlight the importance of monitoring larger areas to detect fish that emigrate from the study reach. Sampling large areas is inherently difficult but may be necessary to accurately measure movement (Albanese et al. 2003). Failure to search outside of designated study areas will often discount mobile individuals, which can account for a small proportion of individuals that are a potentially important component of population processes (Smithson and Johnston 1999; Rodriguez 2002). Thus, as with salmonids (Rodriguez 2002), analytical and conceptual models of movement for small, benthic fishes should account for population heterogeneity with both sedentary and mobile individuals.

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Summer microhabitat partitioning by different size classes of masu salmon (*Oncorhynchus masou*) in habitats formed by installed large wood in a large lowland river

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Abstract: Different size classes of masu salmon (*Oncorhynchus masou*) were partitioned in three-dimensional space in habitats created by artificially installed large wood (LW) structures in a large river. Fish >300 mm in size (L-sized) returning from the ocean distinctly occurred in sheltered areas near the riverbed, which had a moderate current velocity and contained large root wads or tree trunks; 140–200 mm (M-sized) and 100–120 mm (S-sized) fish selected deep areas of high velocity current adjacent to LW structures; ≤80 mm fish (SS-sized) were most common in the lower depth layers throughout all LW habitats, including shallow areas with moderate currents where LW structures blocked the fast currents. Some SS-sized fish used cover areas provided by branches and leaves. Masu salmon abundance in all size classes combined was greater in habitats with LW structures than in habitats without them. Our study suggests that the natural recruitment of whole trees from the riparian zone or artificial placement of whole trees will have a profound effect on creating salmonid habitats in large rivers.

Résumé : Les différentes classes de taille de saumons masou (*Oncorhynchus masou*) se répartissent dans l'espace tridimensionnel des habitats créés par l'installation d'importantes structures de bois (LW) dans une grande rivière. Les poissons >300 mm (de taille L) qui reviennent de l'océan se retrouvent de façon caractéristique dans les zones protégées près du lit de la rivière qui possèdent un courant moyen et de gros amas de racines ou des troncs d'arbres. Les poissons de 140–200 mm (de taille M) et de 100–120 mm (de taille S) choisissent les zones profondes à courant rapide adjacentes aux structures LW. Les poissons ≤80 mm (de taille SS) sont plus communs dans les couches les plus profondes dans tous les habitats de LW, mais aussi dans les zones peu profondes à courant modéré où les structures LW bloquent les courants rapides. Quelques poissons de taille SS utilisent les zones de couvert formées par les branches et les feuilles. L'abondance des saumons masou, toutes classes de taille combinées, est plus importante dans les habitats contenant des structures de LW que dans ceux qui n'en ont pas. Notre étude laisse croire que l'apport naturel d'arbres entiers provenant du rivage ou l'addition artificielle d'arbres entiers a un effet profond en créant des habitats à salmonidés dans les grandes rivières.

[Traduit par la Rédaction]

Introduction

Large wood (LW), which originates in forests and is transported into rivers, can have a significant influence on channel form (Nakamura and Swanson 1993, 1994; Montgomery et al. 2003), water velocity (Mutz 2003), dynamics of nutrients and organic matter (Bilby 2003; Seo et al. 2008), and the ecology of aquatic invertebrates (Benke and Wallace 2003) and fish (Dolloff and Warren 2003; Zalewski et al. 2003) in the processes of decomposition and transport toward the ocean. In the context of habitat rehabilitation for salmonids, which are an important aquatic resource for many countries in the northern hemisphere, many researchers have investigated the effects of LW placement on salmonids in rivers. For example, artificially installed LW form pools that provide an important habitat for salmonids (Riley and Fausch 1995; Roni and Quinn 2001) and become spring and winter cover, functioning as refugia for salmonids from predators and fast current velocities (Shirvell 1990; Zika and Peter 2002). However, most research on salmonid habitat rehabilitation using LW has been conducted in small streams, and few studies have evaluated its use in large rivers (Za-

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lewski et al. 2003), mainly because of the difficulties in examining fish habitats effectively in deep and fast currents (Murphy et al. 1989; Beechie et al. 2005).

Habitat requirements by salmonids vary with life stages. Previous studies in small streams in Hokkaido, northern Japan, found that different life stages (size classes) of masu salmon (*Oncorhynchus masou*) showed different habitat preferences and different use of wood as cover. Inoue and Nakano (1999) showed that masu salmon parr (fork length (FL) 90–150 mm) aggregated in pools, whereas fry (FL 40–70 mm) were distributed in other channel units (riffles and glides) and pools during summer. Miyakoshi et al. (2002) showed that during fall, masu salmon abundance at age 0 (FL 74–89 mm) and age 1 and older (FL 93–115 mm) showed no and positive correlations with abundance of cover (e.g., woody debris), respectively. Large adult masu salmon (FL 300–422 mm) returning from the ocean preferred specific habitats during summer: large pools characterized by greater depth and large cover (e.g., coarse woody debris), but with moderate current velocity (4.3 cm·s^{-1}) (Edo and Suzuki 2003).

From spring through fall, masu salmon at different life stages coexist in the same reaches of large rivers. Masu salmon juveniles reside in freshwater for the first year after emergence. Anadromous masu salmon migrate to the ocean after this period, whereas stream-resident types remain in freshwater. After spending 1 year in the ocean, anadromous masu salmon return to their natal rivers in spring and reside in freshwater until the fall for spawning. Because of their life history, juvenile and adult masu salmon of both stream-resident and the anadromous types occur in large lowland rivers in northern Japan (e.g., Kawaguchi et al. 2005). Therefore, downstream sections of large rivers are important habitats for masu salmon populations.

Therefore, to implement rehabilitation works for masu salmon in large rivers, habitat requirements at multiple life stages should be satisfied. Woody debris apparently plays a key role in creating essential habitats for juvenile and adult masu salmon. Two previous studies in Japan on masu salmon habitat rehabilitation using wood reported positive effects of small log dams on abundance of juveniles (Toyoshima et al. 1996; Yanai et al. 2004). Some research on habitat rehabilitation for other salmonids indicated a close relationship between instream LW and salmonid abundance at multiple life stages (size classes) (Peters et al. 1998; Roni and Quinn 2001; McHenry et al. 2007). However, no studies have examined the effect of habitat structures formed by installed LW on salmonids at multiple life stages in large rivers.

In this study, we examined microhabitat partitioning by different size classes of masu salmon in a large lowland river during summer, focusing on the role of man-made LW structures in creating diverse habitat conditions. The life stages studied ranged from juveniles to adults and consisted of both anadromous and stream-resident types. We also discuss effective LW structure functioning at various life stages and the role of naturally recruited LW in successful salmonid habitat restoration in large rivers.

Materials and methods

Study sites

Our study reach was located in the Shibetsu River in Hokkaido, northern Japan (Fig. 1). The river originates in Mount Shibetsu (1061 m) and flows along 77.9 km (drainage area 671 km^2) to the Okhotsk Sea. Natural conifer and deciduous mixed forest dominates the upper basin, whereas pasture primarily extends over the middle to lower basin.

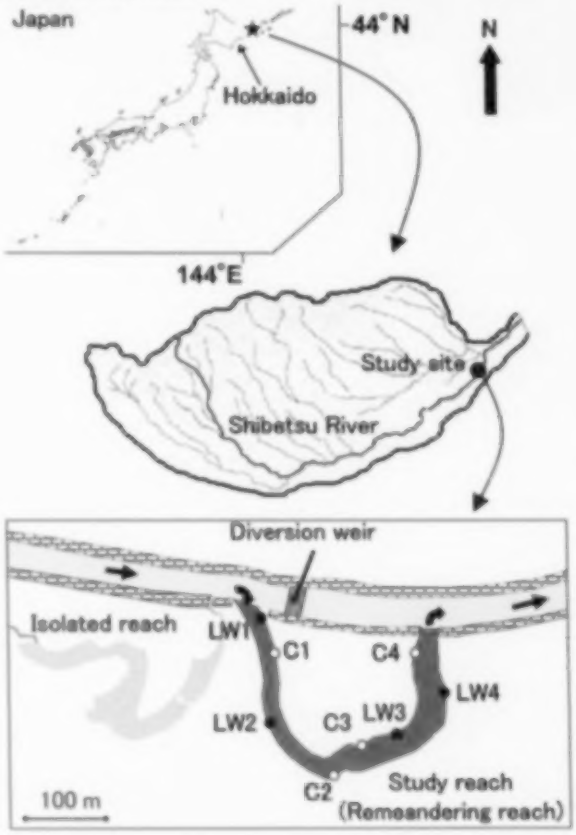
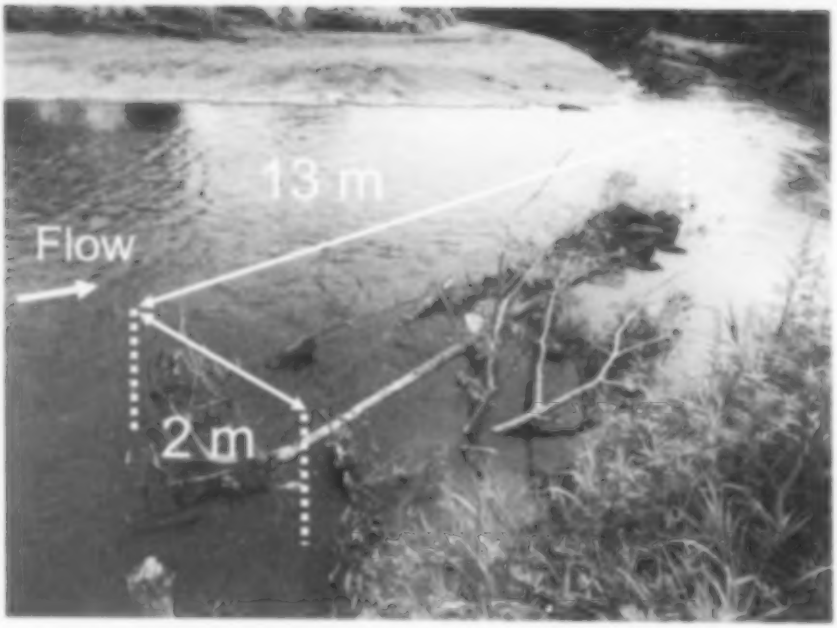


Fig. 1. Location of the Shibetsu River, Hokkaido, Japan, and arrangement of large wood (LW1–LW4) and control (C1–C4) sections in the study reach.

We conducted this study during the summer base-flow period from 23 to 25 July 2005. Water temperature ranged from 15.4 to 17.3 °C. Our study reach, 470 m long and 25–30 m wide at the base flow, was located in the downstream section of the Shibetsu River about 8.5 km upstream from where the river meets the ocean (Fig. 1). The riverbed was composed of sandy gravel. We considered the study reach as a large river in relation to wood dynamics, where the channel width is greater than the length of wood pieces that are actively transported downstream during flooding (Piégay 2003). The mean daily discharge for the whole river at the study site from 1990 to 1999 was ~17 m^3·s^{-1}, ranging from 4 to 293 m^3·s^{-1}. Almost all base flow (13 m^3·s^{-1}) was directed to the study (remeandering) reach by the diversion weir (Fig. 1). The study reach was an impounded historic meander bend that had been artificially disconnected from the river by channel straightening (Nagayama et al. 2008; Nakamura et al. 2008). Reconnecting the old meander bend with the main channel as a remeandering experiment in

Fig. 2. Photograph of large wood section 2 (LW2).



2002 restored discharge to the reach. The remeandering restoration program will be extended to the lower section of the river (Nagayama et al. 2008).

Eight survey sections were selected at lateral scour pools (mean depth 78.6–121.8 cm) in the study reach, where water currents encounter the stream bank (Bisson et al. 2006) (Fig. 1). Each section was about 14 m long and 3 m wide. We made four sets of artificial LW structures about 13 m long and 2 m wide and installed one set in each of the four sections (LW1–LW4) (Figs. 1 and 2). The remaining four survey sections where LW structures were not installed were set as control sections (C1–C4) (Fig. 1). These four LW and four control sections were evenly distributed over the entire study reach. Prior to the LW installation, masu salmon abundance was not significantly different between the LW and the control sections (Y. Kawaguchi, unpublished data). Each set of LW structures consisted of two deciduous trees (tree height 8–10 m), one conifer tree (tree height 10–13 m), and one large mass of root wads (weight about 800 kg). These materials were cabled tightly together, anchored to the shore by wires, and laid parallel to the stream current in the channel margin in June 2004 (Fig. 2). The root wads were placed at the upstream end of the LW structures, and the trees were left under floating conditions immediately downstream from the root wads (Nagayama et al. 2008).

The seven salmonid species observed in the lower Shibetsu River are masu salmon, rainbow trout (*Oncorhynchus mykiss*), chum salmon (*Oncorhynchus keta*), pink salmon (*Oncorhynchus gorbuscha*), Dolly Varden (*Salvelinus malma malma*), white spotted char (*Salvelinus leucomaenis*), and Sakhalin taimen (*Hucho perryi*) (Yamashiro 1965; Kawaguchi et al. 2005; Akita et al. 2006; Makiguchi et al. 2007, 2008). Dolly Varden, white spotted char, and rainbow trout are not very abundant. Sakhalin taimen is in danger of extinction in the river basin, with very few observations of individuals and the absence of spawning redds. In summer, no pink or chum salmon inhabit the river because juveniles migrate to the sea soon after emerging from spawning redds during spring. Masu salmon are dominant throughout the year in the lower Shibetsu River. Masu salmon are distributed in far eastern Asia in the Pacific Rim region (Kato 1991). In Japan, this species has stream-resident and anadromous types. Masu salmon juveniles and adults of both types coexist in the downstream section of the Shibetsu River from spring through fall.

Masu salmon population survey

We divided the LW and the control sections into individual rectangular cells 1 m wide × 2 m long as fish observation units (Fig. 3). We established 2 m spaced transect lines perpendicular to the stream current and divided each space between the two adjoining lines into 1 m wide cells (Fig. 3). Before undertaking the physical habitat survey, the number, total length (TL), and focal point depth of individual masu salmon in all cells were visually estimated by a diver using a compressed-air tank. The clarity of the water was about 2 m during the underwater observation. To collect reliable data, even in fast and deep water, the diver entered the water more than 20 m upstream of the uppermost end of each section. The diver then drifted downstream in a straight line and observed fish while reducing drifting speed with a fixed rope. The diver maintained position at the midchannel side of the section and dived down when observing deep areas of more than 1 m and shaded areas under the LW structures. This downstream drifting survey did not scatter fish (diver's observation). When the diver observed masu salmon, he rose up to the water surface and informed another investigator on the riverbank about TL and focal point

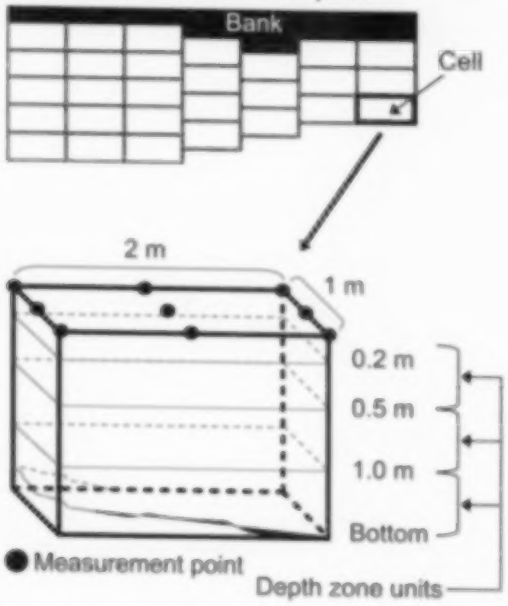


Fig. 3. Planar view of a study section divided into fish observation cells and a diagram of a cell vertically layered with depth zone units. The solid circles are measurement points.

depth of fish while pointing out its planar position. Based on markers established at 2 m intervals along the riverbank, the investigator determined to which cell on the prepared sectioned map the diver pointed and recorded fish data on it. Even if no cells were readily visible because of lateral obstruction by the LW structures, the diver continued the downstream drifting survey. After completing the first pass, the diver returned to these cells for a second observation by approaching from downstream of the cells. The diver was experienced in underwater visual estimation of fish lengths and estimated the TL of each fish to the nearest 10 mm. The underwater observation was consistently conducted by the same diver. During the underwater observation, no predatory fish on masu salmon were observed.

Habitat survey

To characterize the physical environment, we measured depth, current velocity, and cover type. Depth was measured at the center and at the four corners of each cell and the midpoint of each side (nine measurement points in total; Fig. 3). Current velocity was measured at the bottom of each of the nine points defining the cell and at various depths, depending on each point's maximum depth as follows: 0.2 m for a maximum depth of ≤0.5 m; 0.2 m and 0.5 m for a maximum depth of 0.5–1.0 m; 0.2 m, 0.5 m, and 1.0 m for a maximum depth of >1.0 m. Depth was measured from the water surface. Because of water surface turbulence, we measured the current velocity at a depth of 0.2 m for surface velocity. Current velocity for each point at a given depth was determined as the mean of three measurements, which were averaged over a 2-s period with a portable current meter (model CR-7WP; Cosmo Riken Inc., Kashihara, Japan).

The water column of each cell was vertically divided into one, two, or three depth zone units of 0–0.5 m, 0.5–1.0 m, and >1.0 m, depending on the water depth of the cell (Fig. 3). At each depth zone unit, instream LW structures in all LW sections were visually classified into four cover types (no cover, sparse, dense, and large). Sparse or dense cover type was determined by the denseness of branches and leaves. Large cover type represented large root wads or trunks. This cover survey was not conducted in any control sections because cover structures were few.

Data analysis

One-way analysis of variance (ANOVA) was used to examine the difference in masu salmon density of all size classes combined between the LW ($N = 4$) and the control ($N = 4$) sections. Masu salmon density was calculated by the number of fish divided by the area in each section, and $\log_{10}(x + 1)$-transformed to stabilize variances and improve normality.

To characterize the habitat for masu salmon in the horizontal plane, we first classified all cells into habitat types based on three variables: current velocity, current variability, and depth. Current velocity and depth for each cell were estimated as the mean of all measurements for the cell. Current variability was expressed as the coefficient of variation of all velocities measured for each cell. To identify predominant habitat types, we performed the Ward's hierarchical method of cluster analysis (van Tongeren 1995) on all cells ($N = 153$) in the LW and control sections combined. For this analysis, the squared Euclidean distance measure was used. The number of clusters was determined by finding the merging step that had a noticeable decrease in the similarity coefficient in a process of clustering. Current velocity, current variability, and depth were $\log_{10}(x + 1)$-transformed to stabilize variances and improve normality and standardized to correct scale differences in measurement before the cluster analysis.

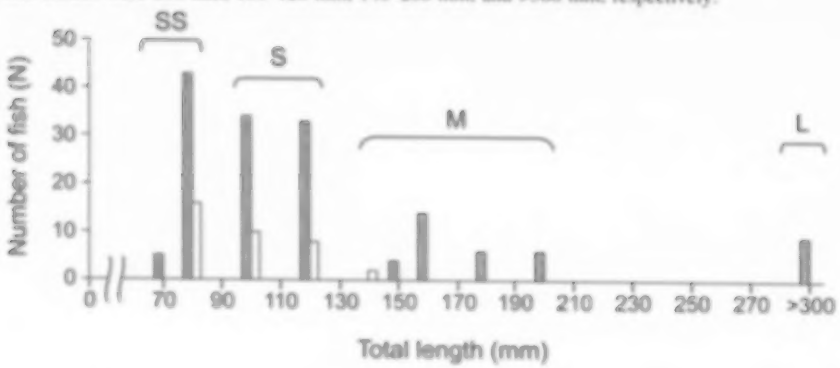
Subsequently, to estimate relative preferences for each habitat type by each of the different size classes of masu salmon, Manly's α, also known as Chesson's index (Chesson 1978; Manly et al. 2002), was used:

$$\alpha_i = \frac{r_i/n_i}{\sum_{j=1}^{m} r_j/n_j}, \qquad i = 1, 2, \ldots, m$$

where α_i is the preference value for an ith habitat type (of m types) at a particular study section, and r_i and n_i are the proportions of the cells of the ith habitat type in all cells used by the fish and in all cells within the study section, respectively. The value ranges from 0 to 1. Generally, a value of $\alpha_i > 1/m$ indicates relative preference and a value of $\alpha_i < 1/m$ indicates relative avoidance. Habitat type preference was examined separately in the LW and the control sections. This approach using the cluster analysis and the preference index can examine the responses of fish to combinations of all habitat variables with reference to spatial distribution (Inoue and Nunokawa 2002).

To examine the vertical utilization of the water column by masu salmon, the distribution of the relative focal point depth (RFPD) was compared among different size classes

Fig. 4. Abundance of four size classes of masu salmon in large wood (LW) (solid bars) and control (open bars) sections. Total lengths (TL) for SS, S, M, and L size classes were ≤80 mm, 100–120 mm, 140–200 mm, and >300 mm, respectively.



using Kolmogorov–Smirnov tests in each LW and control section. To adjust for multiple comparisons, we employed the Bonferroni method (Sokal and Rohlf 1995). The RFPD was expressed as a proportion of the focal point depth to the mean depth of the cell in which the masu salmon were present.

Preferences in LW cover types determined in each depth zone unit were also calculated using the above formula of Manly's α. α_i is the preference value for an ith cover type (of m types; $m = 4$ in this case) at the LW sections, and r_i and n_i are the proportion of the ith cover type in depth zone units used by fish and that in all depth zone units within the LW sections, respectively. Cover preference was examined only in the LW sections.

Results

Abundance of different size classes of masu salmon

Based on the frequency distribution of the TL of masu salmon observed in the LW and the control sections, fish were classified into four size classes: SS, ≤80 mm; S, 100–120 mm; M, 140–200 mm; and L, >300 mm (Fig. 4). L-sized fish were age-2 or age-3 adults of the anadromous type returning from the ocean in the spring. S- and M-sized fish were age-1 and age-2 fish consisting of parr of the anadromous and resident types and adults of the resident type. SS-sized fish consisted of age-0 and age-1 parr of both types. We distinguished the SS size class (TL ≤80 mm) because juvenile masu salmon less than 70 mm in FL showed different habitat selection from fish greater than 90 mm in FL (Inoue and Nakano 1999).

In all LW sections, the abundances of SS-, S-, M-, and L-sized fish were 48 (0.28·m^{-2}), 67 (0.39·m^{-2}), 30 (0.18·m^{-2}), and 9 (0.05·m^{-2}), respectively, whereas abundances for the control sections were 16 (0.12·m^{-2}), 18 (0.13·m^{-2}), 2 (0.01·m^{-2}), and 0 (0·m^{-2}) (Fig. 4; Table 1). Masu salmon density in all size classes combined was significantly higher in the LW sections than in the control sections (one-way ANOVA, $F_{[1,6]} = 15.75$, $P = 0.007$).

Because the numbers of M- and L-sized fish in the control sections were only 2 and 0, respectively, the following analyses regarding habitat and cover preferences were not conducted for these size classes in the control sections.

Table 1. The number of masu salmon of the different size classes in four large wood (LW) and four control sections.

	LW section				Control section			
Size class	LW1	LW2	LW3	LW4	C1	C2	C3	C4
SS	20	14	10	4	3	9	4	0
S	25	17	17	8	2	14	0	2
M	7	3	17	3	0	2	0	0
L	3	3	0	3	0	0	0	0
Total	55	37	44	18	5	25	4	2

Note: SS, ≤80 mm total length (TL); S, 100–120 mm TL; M, 140–200 mm TL; L, >300 mm TL.

Hydraulic conditions in the LW and control sections

Cluster analysis identified three habitat types (types 1–3) (Table 2). Type 1 habitat was characterized by the shallowest water and the lowest current velocity and current variability of all types (Table 2). Type 2 habitat was characterized by the largest current variability and a large depth, but with a moderate current velocity (Table 2). Type 3 habitat was the deepest water, with the fastest currents and relatively low current variability (Table 2).

Type 1 habitat was distributed just downstream from the LW structure or between the LW structure and the bank (Fig. 5*a*). Type 1 habitat was also distributed in river margins in the control sections (Fig. 5*b*). Percent area of type 1 habitat tended to be lower in the control sections than in the LW sections (Table 3). Type 2 habitat tended to coincide spatially with the LW structures in the LW sections (Fig. 5*a*), whereas in the control sections, type 2 habitat was partially distributed along the riverbank and laterally midway of the sections (Fig. 5*b*). Percent area of type 2 was higher in the LW sections than in the control sections (Table 3). Type 3 habitat was found partially on the mid-channel sides of the LW structures in the LW sections (Fig. 5*a*), whereas it was broadly distributed in the control sections (Fig. 5*b*). In the LW sections, the distributions of type 3 habitat corresponded to the convergence of mid-channel currents that are created by the LW structures. Percent area of type 3 habitat was higher in the control sections than in the LW sections (Table 3).

Table 2. Mean and standard deviation of habitat variables for each habitat type.

	Habitat type		
	Type 1	Type 2	Type 3
Depth (cm)	34.3±13.0	70.2±18.9	82.1±22.4
Current velocity ($cm \cdot s^{-1}$)	10.9±7.3	15.2±5.3	44.1±13.0
Current variability (%)	45.7±12.5	67.9±18.5	50.7±12.6

Table 3. Area and percent area of each habitat type in large wood (LW) and control sections (mean ± standard error).

	LW section ($N = 4$)	Control section ($N = 4$)
Area (m^2)	42.5±7.0	34.0±5.8
Percent area		
Type 1 (%)	15.5±10.2	8.1±6.7
Type 2 (%)	42.2±4.3	17.9±6.9
Type 3 (%)	42.3±10.1	74.0±9.4

Planar habitat of masu salmon in the LW and control sections

Masu salmon in the SS size class did not show any distinct preference values for habitat types in either the LW or control sections (Fig. 6). This indicates that SS-sized fish occurred in all the habitat types, including type 1 habitat with uniform and moderate current conditions, which was completely avoided by S-, M-, and L-sized masu salmon (Fig. 6). Masu salmon in the S size class selected type 2 and type 3 habitats in the LW sections (Fig. 6*a*), but only type 3 habitat in the control sections (Fig. 6*b*). M- and L-sized masu salmon in the LW sections showed strong preferences for type 3 and type 2, respectively (Fig. 6*a*), although the sample size of L-sized fish was small ($N = 9$).

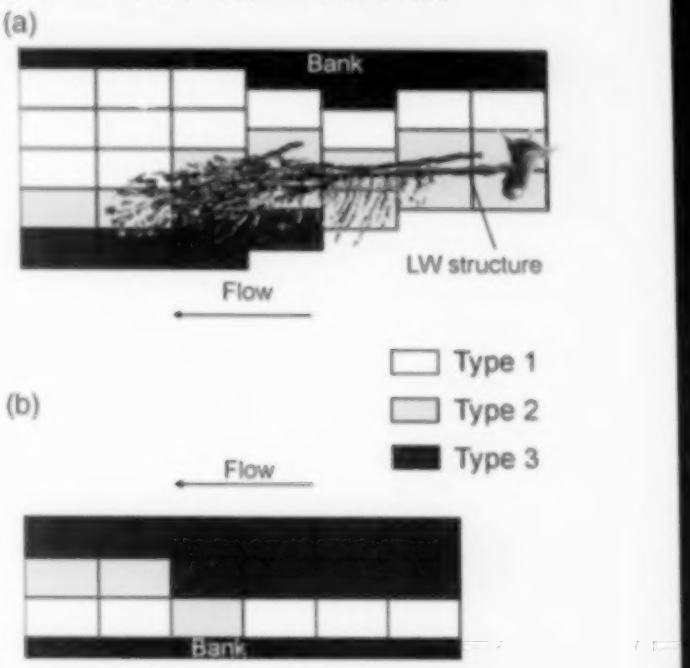
Vertical positions of masu salmon in the LW and control sections

The results of the Kolmogorov–Smirnov tests showed significant differences in distributions of RFPD between L size class and other size classes in the LW sections ($P < 0.05$). All L-sized masu salmon ($N = 9$) were present on or near the bottom (RFPD = 1) (Fig. 7*a*). There was no significant difference in the distribution of RFPD among the SS, S, and M size classes. These size classes were mainly found in the range of 0.66 to 0.99 in RFPD, which occurred in the lower depth layers in the water column (Fig. 7*a*). In the control sections, there was no significant difference in distributions of RFPD between SS and S size classes. These size classes were distributed across a broad range of water depths (Fig. 7*b*).

Response of masu salmon to the LW cover

Masu salmon in the L size class showed a distinct preference for large cover such as root wads or trunks, whereas fish in other size classes did not show any significant preferences for any particular cover type (Fig. 8). However, preference values of SS-sized fish for the three cover types (no cover, sparse, and dense) was about three times greater than that for the large cover type.

Fig. 5. Planar distribution pattern of three habitat types in (*a*) large wood section 2 (LW2) and (*b*) control section 4 (C4).



Discussion

It is a global and local concern that segments of high-order large rivers have been ecologically degraded because of their location in areas with intensive human activities (Tockner and Stanford 2002; Aarts et al. 2004; Yoshimura et al. 2005). The channel regulation in large rivers is generally associated with removal of riparian forest, which results in loss of LW recruitment into rivers (Nagasaka and Nakamura 1999; Nakamura and Swanson 2003). Our study revealed that in a large river, four size classes of masu salmon are partitioned in three-dimensional space within habitats created by artificially installed LW structures. The abundance of masu salmon in all size classes combined is higher in the LW habitat than in habitat without LW. These results indicate that man-made LW structures in large rivers can increase local density of masu salmon through creation of habitats suitable for multiple life stages.

Use of LW habitat by different size classes of masu salmon in a large river

Masu salmon >300 mm (L-sized), which were adults returning from the ocean, exhibited distinctive space and cover preferences in the LW sections. They inhabited the bottom of type 2 habitat, characterized by deep water but with moderate current velocity, with large cover formed by root wads or trunks. Many researchers have reported similar results: large adult salmonids use deep pools sheltered with large cover materials such as bedrock ledges, boulders, and coarse woody debris (Nakamoto 1994; Baigún 2003; Edo and Suzuki 2003), because such habitats are advantageous

Fig. 6. Relative preferences for habitat types described by Manly's α by different size classes of masu salmon in (*a*) large wood (LW) sections and (*b*) control sections. Higher values indicate stronger preferences for the types. Size classes: SS, ≤80 mm total length (TL); S, 100–120 mm TL; M, 140–200 mm TL; L, >300 mm TL.

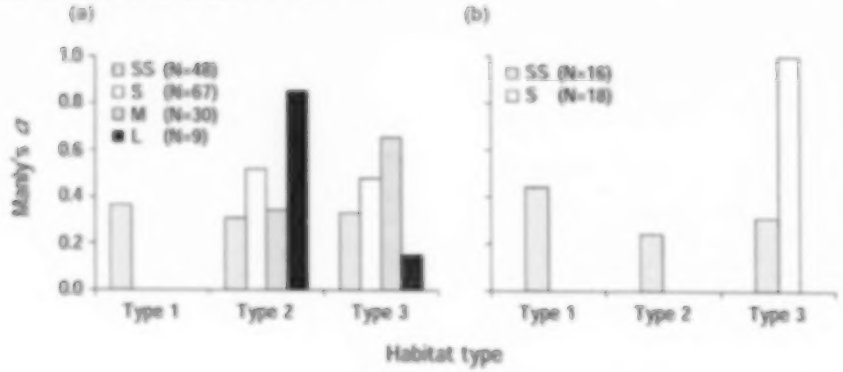


Fig. 7. Frequency distribution of relative focal point depth (RFPD) of masu salmon at different size classes in (*a*) large wood (LW) sections and (*b*) control sections. The value of RFPD from 0 through 1 was classified into four ranges to indicate the upper (<0.33), middle (0.33–0.65), lower (0.66–0.99), and bottom (1) depth layers in the water column. Size classes: SS, ≤80 mm total length (TL); S, 100–120 mm TL; M, 140–200 mm TL; L, >300 mm TL.

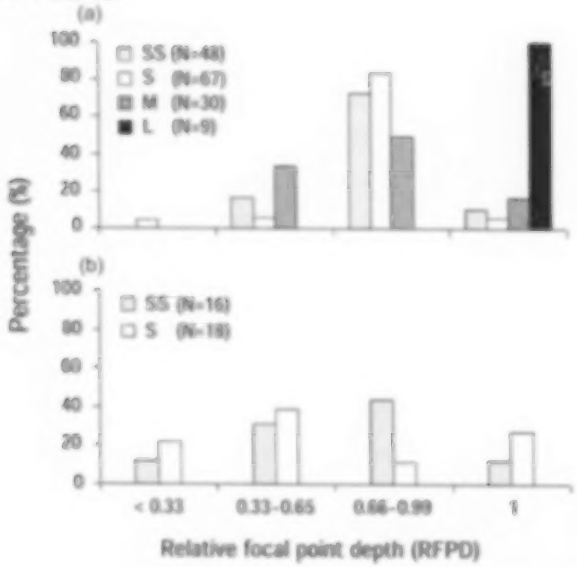
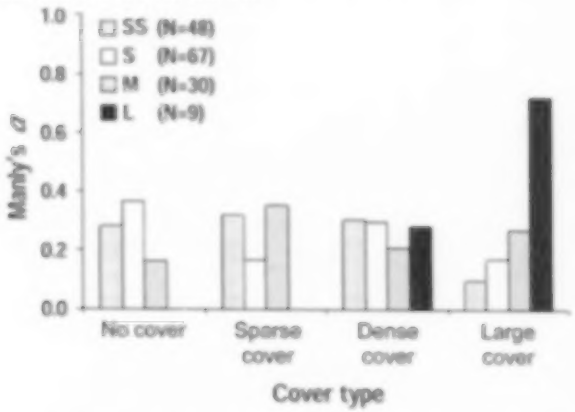


Fig. 8. Preferences for cover types shown by different size classes of masu salmon in large wood (LW) sections. Higher values indicate stronger preferences for the cover types. Size classes: SS, ≤80 mm total length (TL); S, 100–120 mm TL; M, 140–200 mm TL; L, >300 mm TL.



in reducing energy expenditure for exposure to fast velocities and potential predation risk. Adult masu salmon need to reduce their energy expenditure because they cease feeding after returning to the river in spring (Tago 2000). Also, their predation risk decreases in deeper water compared with shallower areas (Harvey and Stewart 1991). In the lower section of the Shibetsu River, brown bears (*Ursus arctos*), red foxes (*Vulpes vulpes schrencki*), and Blakiston's fish owls (*Ketupa blakistoni*) are considered potential predators. In the control sections, however, colonization by L-sized masu salmon could not occur because of the absence of large cover. Because of the small sample size of L-sized masu salmon in the present study, additional data are needed to confirm the habitat preferences by large adult masu salmon.

Masu salmon in the M (140–200 mm) and S (100–120 mm) size classes in the LW sections and in the S size class in the control sections preferred type 3 habitat. Type 3 habitat was characterized by deep water, fast current velocities, and little cover. This indicates that M- and S-sized masu salmon (large parr) may selectively occupy spaces that provide a feeding advantage. Most drifting invertebrates are transported by fast water currents (Mitchell et al. 1998). These advantageous foraging areas are exclusively occupied by large parr (Fausch 1984; Nakano 1995).

Although type 3 habitat was more abundant in the control sections than in the LW sections, abundances of S- and M-sized fish were greater in the LW sections than in the control sections. This indicates that the quality of type 3 habitat would be different between the LW and control sections. Mitchell et al. (1998) reported that larger Atlantic salmon

parr (FL >100 mm) were found in areas close to instream structures, where both water currents and invertebrates are concentrated. Moreover, abundance of juvenile sea trout can be high in the microhabitats collecting abundant drifting food from the adjacent fast water currents (Elliott 2002). LW structures situated close to fast water currents will provide a suitable feeding area for S- and M-sized masu salmon, while providing quick access to cover (diver's observation).

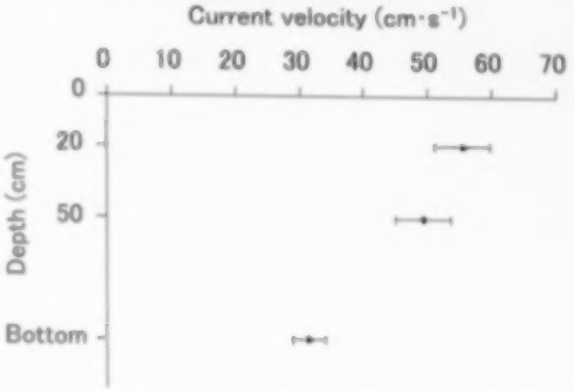
Similar preference by masu salmon parr for deep and fast water with few cover materials has been also detected from cell-based (cell size 1 m × 2 m) analysis in a small stream during fall (Nagayama and Nakamura 2007). However, according to Inoue and Nakano (1998), abundance of masu salmon parr was positively correlated with cover area at reach and channel-unit scales in small streams during summer. Similarly, at the section scale in our study, abundances of S- and M-sized masu salmon parr were positively associated with installed LW. These indicate that during summer and fall, depth and current velocity can be important determinants for masu salmon parr in small spatial scales, whereas cover can be a significant variable at larger spatial scales such as channel unit and reach.

Masu salmon in the S size class also preferred type 2 habitat adjacent to type 3 habitat in the LW sections, although M-sized masu salmon showed no significant preference. This difference in habitat preference might be caused by competition for foraging areas between S- and M-sized fish. According to a study in a small stream, when large masu salmon parr used the middle or upper depth layers as an optimal foraging area, small parr required much larger foraging areas in the lower depth layers, because of dominance hierarchies depending on fish size (Nakano 1995).

Masu salmon in the S and M size classes were mainly distributed in the lower depth layers within deep water columns in the LW sections. However, according to a study in a small stream, large masu salmon parr used the middle or upper depth layers as optimal foraging areas (Nakano 1995). In a large river section with faster overall currents, even large parr (S and M size classes) might have difficulty using the middle and upper depth layers because of energy constraints. In our LW sections, water current velocity in type 3 habitat preferred by S- and M-sized fish was stratified by depth, with velocity decreasing from the surface to the bottom (Fig. 9). To enhance energy efficiency, S- and M-sized fish might avoid the middle and the upper layers with fast water current velocity and mainly use the lower depth layers with relatively moderate current velocity (Fig. 9).

Masu salmon in the SS (≤80 mm) size class were widely distributed in the lower depth layers throughout all LW sections. One reason for this distribution may be that SS-sized fish were the weakest individuals in competing for foraging areas (Nakano 1995). As a result, the smallest SS-sized fish might widely use lower depth layers of type 3 and adjacent type 2 habitats. Only SS-sized fish also used shallow areas with a moderate current velocity (type 1), where LW structures blocked fast currents. The blocking effect by LW structures allows small fish that have weak swimming performance to stay in type 1 habitat. Moore and Gregory (1988) reported similar results for trout fry (TL <80 mm), which preferred shallow stream margins with moderate current velocity in a third-order stream. In our study, SS-sized masu salmon showed preferences for sparse and dense cover types consisting of branches and leaves in the LW sections, and these complex covers provided small spaces with moderate current for SS-sized fish. Thus, availability of diverse microhabitats might contribute to a higher abundance of SS-sized fish in the LW sections than in the control sections.

Fig. 9. Mean (± standard error) current velocities at each depth in type 3 habitat with ≤100 cm depth in large wood (LW) sections. Type 3 habitat was characterized by the deepest water, fastest currents, and relatively low current variability.



Implication for masu salmon habitat rehabilitation in large rivers

We found three features of LW structures that should contribute to improvement in masu salmon habitat in large rivers. First, large root wads and trunks provide important habitat cover for L-sized masu salmon returning from the ocean, as shown by their distinctive preference for areas with large wood and associated cover. Second, the long structure of LW with large root wads at the upstream end of the structure can create two different habitat types, one preferred by M- and S-sized fish and another preferred by SS-sized fish. The long structure generates areas of aggregated water current on the midchannel side of the LW, whereas areas of moderate velocity occur on the other side (streambank side). The third characteristic is a complex cover structure consisting of branches and leaves, which contain spaces that can be used by SS-sized fish. These structural features may have relevance to understanding the role of wood for multiple life stages of other salmonid species.

The above features of installed LW structures were composed of all parts of a tree, rootwad and trunk with branches and leaves. Many previous studies on salmonid habitat rehabilitation have used a part of a tree, such as the trunk (Riley and Fausch 1995), root wad (Shirvell 1990), trunk with branches and leaves (Zika and Peter 2002), and trunk with root wad (McHenry et al. 2007). To maximize the efficiency of LW placement for salmonid habitat rehabilitation in large rivers, we propose the placement of whole trees with root wad, trunk, branches, and leaves or combined LW structures with all those parts.

The present study indicates that man-made LW structures can improve salmonid habitat in large rivers. However, these

artificial structures would not function over a long time frame because of washout during floods, sedimentation, and biological and physical decay. We recognize that artificial LW structures can function at local scales and for a short time frame, and therefore it would be economically unrealistic to introduce these structures to an extensive segment of modified large rivers. What is essentially important in long-term fish habitat restoration is to restore natural processes of wood recruitment and routing. Fluvial and climatic disturbances stimulate wood supply from riparian forest into streams and rivers and transport it downstream (Nakamura and Swanson 2003), which is a sustainable, dynamic process creating fish habitat associated with wood at a basin scale. Therefore, as source areas of wood recruitment, riparian forests should be preserved. To a certain extent, we should allow natural migration of channel course and erosion of riverbanks for wood recruitment. The diverse geomorphic surfaces developed by channel dynamics may function to retain more large wood within rivers. In conclusion, the artificial wood installation should be implemented as an interim habitat management method in restoration programs that ultimately aim for rejuvenation of natural dynamic processes at a basin scale.

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Physical and ecological response to disturbance by gravel mining in a large alluvial river

Laura L. Rempel and Michael Church

Abstract: The role of sediment transport during high flows for restoring fish habitat was demonstrated following an experimental gravel removal from Fraser River, British Columbia, Canada. Dry bar scalping 69 000 m^3 of river sediment left a topographically simple removal area with a loose surface of gravel and sand. Two subsequent, below-average floods yielded no gravel replenishment but restored substrate grain size and some topographical complexity at the habitat scale. A third, above-average flood replenished 31% of the removal volume. High-elevation bar area, which provides fish habitat at high flows, remained 25% smaller after the three floods. Effects of mining on the fish community could not be confirmed. Benthic invertebrates recolonized the removal site immediately after mining, and differences in community composition compared with three reference sites disappeared during the first flood. Results suggest that physical changes due to this mining operation fell within the range to which local aquatic populations are accustomed during flooding, because the ecological response was modest and short-lived. Despite an extensive sampling program, inherent variability in the biological data reduced statistical power to detect an effect. Monitoring programs to support adaptive management of river fisheries will require substantial investment and planning to yield definitive results.

Résumé : Après une extraction expérimentale de gravier dans le Fraser, la Colombie-Britannique, Canada, nous avons démontré le rôle du transport des sédiments pendant les forts débits dans la restauration des habitats des poissons. Le prélèvement par décapage de 69 000 m^3 de sédiments sur les barres sèches de la rivière a laissé une zone d'extraction à topographie simple avec une surface lâche de gravier et de sable. Deux crues subséquentes, de débit inférieur à la moyenne, n'ont pas rapporté de gravier, mais elles ont restauré la taille des particules du substrat et rétabli une partie de la complexité topographique à l'échelle de l'habitat. Une troisième crue, plus importante que la moyenne, a rétabli 31 % du volume retiré. La zone des hauts-fonds qui représente l'habitat des poissons pendant les forts débits est demeurée 25 % plus petite après les trois crues. Il n'a pas été possible de déterminer l'effet de l'extraction du gravier sur les communautés de poissons. Les invertébrés benthiques ont recolonisé le site d'extraction immédiatement après l'opération minière et les différences de composition de communauté observées en comparaison avec trois sites témoins sont disparues lors de la première crue. Nos résultats laissent croire que les changements physiques causés par cette extraction minière se situent dans la gamme des conditions auxquelles les populations aquatiques locales sont habituées parce que la réaction écologique a été modeste et de courte durée. Malgré un important programme d'échantillonnage, la variabilité inhérente aux données biologiques a réduit notre capacité statistique à détecter des effets. Les programmes de surveillance qui appuient la gestion adaptative des pêches de rivière vont exiger un investissement et une planification importants afin de produire des résultats définitifs.

[Traduit par la Rédaction]

Introduction

The distribution of sediment along stream channels determines the form of the channel, as well as the character of habitats available to aquatic organisms. In steep gradient systems, cobbles and boulders are major structural elements that define habitat boundaries (Halwas and Church 2002), while gravel and smaller-sized sediments are transported downstream. As gradient drops and the flow becomes less powerful, gravel is deposited and accumulates to form bars. The tendency for sediment to accumulate as gravel bars and islands in moderate and low-gradient channels creates outstanding habitat for various fish species and aquatic organisms (Thorp 1992; Johnson and Jennings 1998; Beechie et al. 2005).

River-run sediment is also highly desirable for construction purposes. Alluvial gravel is particularly sought after because of its high quality, often simple removal, and the fact that land-based aggregate is, with population growth, increasingly pre-empted for other uses (Kondolf 1994, 1998). Depending on channel characteristics and jurisdictional regulations, material is either removed by in-stream dredging (e.g., Harvey and Lisle 1998), extracted from off-channel floodplain deposits (e.g., Kondolf 1998), or scalped from dry bar surfaces within the channel during low flow (this study). Volumes extracted from many rivers have greatly exceeded the natural rate of recruitment, and in some cases, severe environmental damage has been reported (e.g., Macdonald 1988; Rinaldi et al. 2005; Rovira et al. 2005).

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Studies characterizing the physical impacts of gravel mining from rivers in the United States (e.g., Collins and Dunne 1990; Kondolf 1994; James 1999) and Europe (e.g., Sear and Archer 1998; Surian 1999) are relatively common. Far fewer studies have examined ecological impacts, despite growing concern that mining damages aquatic habitat. Attempts are made to minimize direct effects, such as the loss of benthic organisms (e.g., Griffith and Andrews 1981) or turbidity causing physiological stress to sensitive fish species (e.g., Berg and Northcote 1985; Shaw and Richardson 2001), through regulations restricting the timing and method of extraction (e.g., Boyd 1975; Meador and Layher 1998).

Indirect ecological effects due to gravel mining, those transmitted through habitat modifications, are likely to have the greatest impact on river ecosystems. But such effects are difficult to detect and characterize. Previous studies examining ecological effects have been carried out at sites with a prolonged history of gravel mining (e.g., Brown et al. 1998). There remains a major gap in our knowledge of the impacts to pristine aquatic habitat and the response of organisms. Gravel mining directly alters channel morphology (Collins and Dunne 1990; Kondolf 1997), thereby affecting flow velocity, water depth, and substrate texture, all of which influence the distribution and abundance of aquatic organisms. Species with specific habitat requirements may disappear from a system where substantial habitat modifications have occurred (Ricciardi and Rasmussen 1999). Other species with greater tolerance to habitat change may persist; however, recurrent habitat alteration will inevitably impact species composition and the productivity of river ecosystems (Benke 1990; Richter et al. 1997).

The objective of our study was to examine the effects of a single, experimental gravel removal on the physical habitat and invertebrate and fish communities in the lower Fraser River, British Columbia. A secondary goal of our study was to evaluate sampling requirements for effect detection in a large river system. Fraser River is unregulated and has a predictable annual hydrograph with snowmelt flooding in late spring that delivers substantial gravel annually to the lower river. Our hypothesis was that habitat alterations and the ecological response due to gravel mining are mediated by this annual flow event.

Materials and methods

Study area

Fraser River drains 232 000 km^2 of south and central British Columbia, Canada. The mainstem river is unregulated, and annual flooding due to snowmelt occurs in spring. At high flows, a large amount of sediment is mobilized and transported from the steep, upper basin, and a sharp decline in gradient in the lower 170 km forces deposition of much of this sediment load. Coarse sediment (gravel, cobble) is deposited within a 50 km reach between Laidlaw and Sumas Mountain (Fig. 1), referred to as the gravel reach, whereas fine sediment (sand, silt, clay) is transported further downstream (McLean et al. 1999). Sediment deposition in the gravel reach creates a wandering channel pattern (Desloges and Church 1989), with gravel bars and islands dividing the flow into multiple channels that shift with bar growth and bank erosion. Average peak flood discharge for the gravel reach (measured at Hope, Water Survey of Canada Stn. 08MF005) is 8766 m^3·s^{-1}, and mean annual flow is 3410 m^3·s^{-1}.

Because of annual gravel deposition, the Fraser River gravel reach has been exploited as a local source of high-quality gravel for many decades. At least 5 million m^3 have been mined since 1964 (averaging 117 000 m^3·year^{-1}; Weatherly and Church 1999), the majority of which was removed by dry bar scalping at low flow in winter. To place this volume in context, annual gravel influx averages 250 000 m^3·year^{-1} to the gravel reach (Ham 2005), while substantially larger volumes of sediment, on the order of 1 million m^3·year^{-1}, are redistributed by natural erosion and deposition processes during spring flooding (Ham and Church 2003). A removal rate of less than 50% the natural influx rate is low relative to most rivers where mining occurs (Collins and Dunne 1990; Rinaldi et al. 2005), and sediment transport processes in the gravel reach are mostly intact.

The Fraser River gravel reach supports 28 native fish species, including endangered white sturgeon (*Acipenser transmontanus*). Its residency in the gravel reach has been a major force behind habitat protection and sustainable management of the reach. The family Salmonidae dominates the faunal assemblage of the gravel reach with 11 species, and the Fraser River exceeds all rivers worldwide in terms of salmonid stock abundance (Northcote and Larkin 1989). Millions of pink salmon (*Oncorhynchus gorbuscha*) spawn within the gravel reach biennially, and large numbers of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) use the reach for rearing.

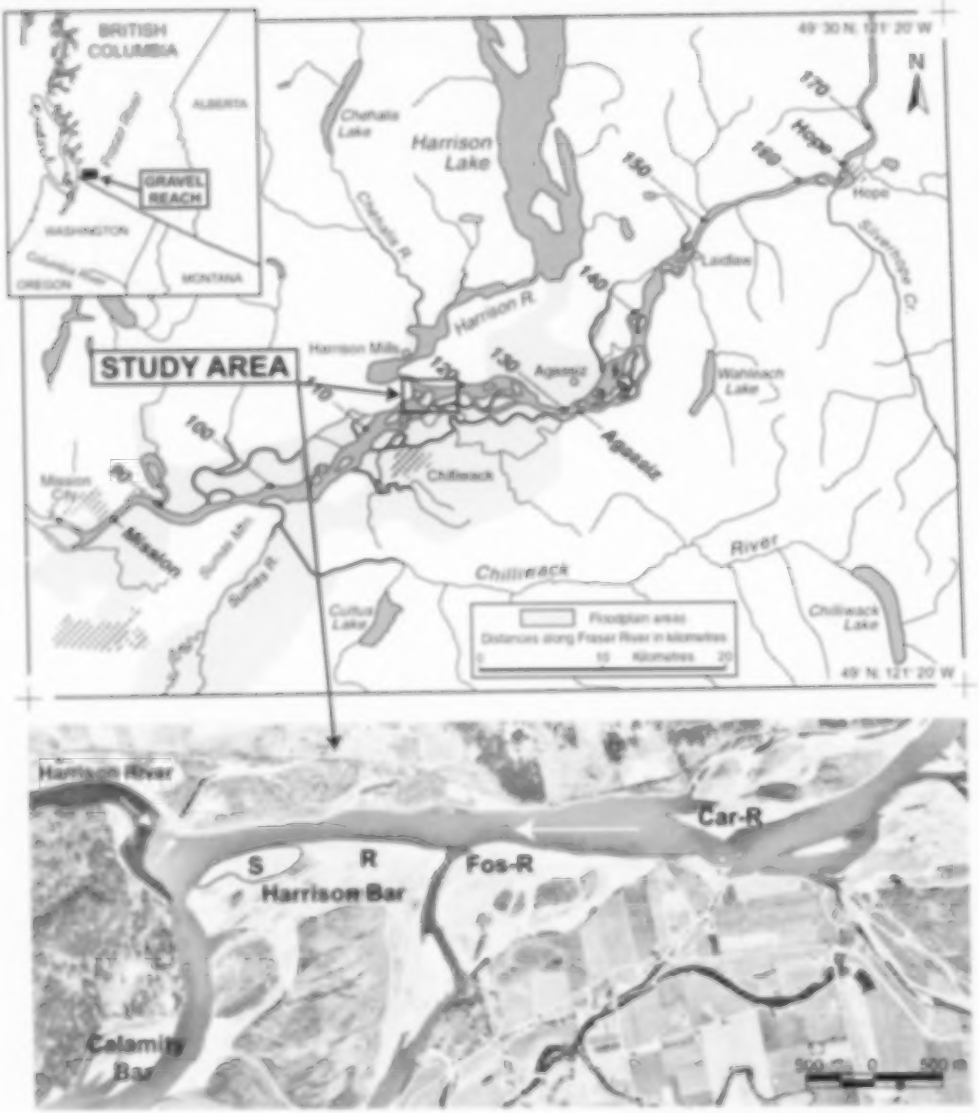
The lower Fraser River valley is also home to 2 million people, and with this concentration of people and investment, there is concern that annual gravel deposition is creating a flood risk. Gravel mining from within the main channel is proposed by river management agencies as a strategy to reduce flood risk, but concern over the potential impacts to fish habitat forced a moratorium on commercial mining from 1998 to 2004 to allow scientific studies to proceed. During the moratorium, one gravel removal (this study) was authorized to assess mining impacts on fish habitat and aquatic organisms.

Gravel mining

Gravel was removed by scalping 69 070 m^3 of dry sediment from lower Harrison Bar (Har-S) in early March 2000. The affected area represented 2% by volume and 15% by surface area of the nonvegetated Harrison Bar (Rice et al. 2008). The contractor followed normal industrial methods in accordance with Fisheries and Oceans Canada regulations. After completion, the site was graded to a 2% slope from the inner bar towards the main channel to ensure positive drainage and no fish stranding.

Upper Harrison Bar (Har-R), along with the lower halves of Carey Bar (Car-R) and Foster Bar (Fos-R), were designated reference sites (Fig. 1). The upstream proximity of reference sites to Har-S meant that they were unaffected by possible changes due to mining, but shared physical characteristics with respect to channel morphology, sediment transport regime, and substrate texture. Carey Bar has no prior history of gravel mining, but a site on Foster Bar located

Fig. 1. Location map of the Fraser River (British Columbia, Canada) gravel reach showing the study area, gravel mining site on Harrison Bar (S), and three upstream reference sites (Har-R, Fos-R, Car-R). Numbers indicate river kilometres measured from the mouth. Photograph taken 27 March 1999.

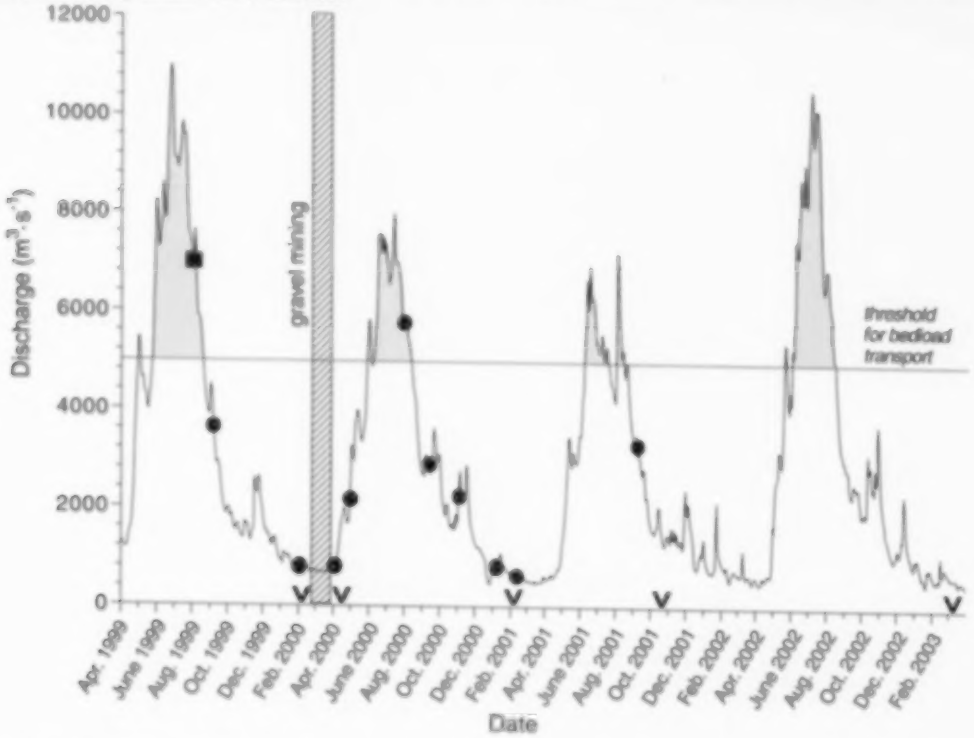


1 km upstream of Fos-R was mined in 1995. Herein, the term scalping is used interchangeably with mining and is defined as the removal by front-end loader of dry sediment from high-elevation areas of gravel bars.

Sampling schedule and design

Sampling began in August 1999 (Fig. 2) as part of a larger study examining the ecology of lower Fraser River. The experimental removal was not then anticipated and pre-removal sampling effort was unequal among reference sites. Mining at Har-S occurred in March 2000 at low flow and sampling after mining extended over 18 months, during which time spring flooding occurred twice. It was only during these annual flood events that any gravel replenishment and habitat recovery at Har-S could take place. Fish sampling at Har-S and the reference sites occurred three times before (August and September 1999, February 2000) and eight times after (April, May, August, September, and November 2000; January, February, and October 2001) mining. Invertebrate sampling occurred twice before (September 1999 and February 2000) and the same eight times after mining. The sampling schedule was designed, within the

Fig. 2. Sampling and topographic survey dates of our study in relation to river discharge measured at Hope, British Columbia (square, fish sampling only; circles, fish and invertebrate sampling; v, topographic survey). Substantial bedload gravel transport occurs at >5000 $m^3 \cdot s^{-1}$. The date that gravel mining commenced is indicated.



constraints of an imposed planning timeline, to coincide with the timing of key life cycle stages of juvenile fish and invertebrates.

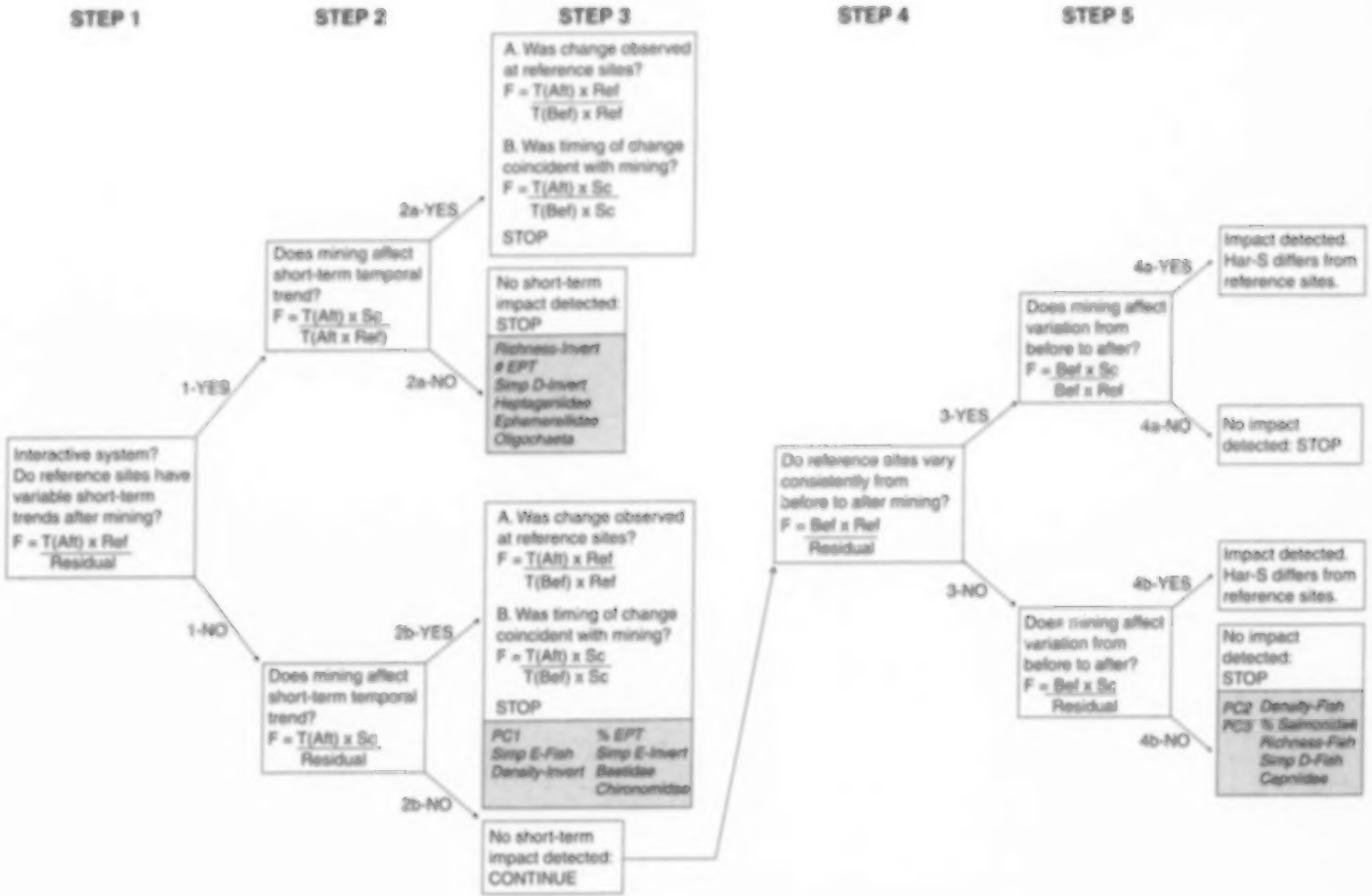
Sampling followed a modified BACI design (before–after control–impact; Stewart-Oaten et al. 1986) with three reference sites rather than a single control site. Including three reference sites leads to an asymmetrical analysis of variance (A-ANOVA; Underwood 1992, 1994, 1997), in which sources of environmental variance are assessed from observations at the multiple reference sites. This statistical design was specifically developed to assess the effects of major anthropogenic treatments in naturally variable systems, which are not often replicated.

A-ANOVA requires multiple reference sites for spatial replication and multiple sampling episodes before and after treatment for temporal replication. Only the reference condition is replicated; hence, the asymmetry. In our study, the difference between Har-S and reference sites prior to mining provided an estimate of the variability in possible outcomes after mining had the removal not occurred, thus making it possible to ascertain what a significant change at Har-S might be. A significant effect was defined as a detectable difference (negative or positive) in the change of measured parameters at Har-S from before to after mining compared with changes from before to after at the reference sites. Thus, a statistical interaction in the difference between Har-S and reference sites from before to after mining was required for the effect of gravel mining to be significant. This modified BACI design allowed for natural differences between the reference sites and Har-S and for changes during the before and after periods that influenced all sites in the same way. The practical mechanics of A-ANOVA are detailed in Underwood (1993) and described briefly herein to facilitate the reporting and interpretation of results for our study.

A series of questions are specified (Fig. 3), which are answered by up to five standard ANOVAs designed to determine if a detectable effect due to gravel mining at Har-S had occurred. Together, the ANOVAs systematically isolate the variance contributed by Har-S, before and after mining, from the total variance in the observations. SYSTAT (version 9.0, SPSS Inc., Chicago, Illinois) was used for the analyses. A-ANOVA was then calculated by subtractions and additions of the component sums of squares. We chose a critical value of $p = 0.05$ as a compromise between inflating the risk of a type I error by performing multiple analyses on the same data set and wanting to ensure that any real effect was detected. Power was estimated following Underwood (1993) for each analysis that failed to detect a significant impact.

If a significant temporal interaction is detected among reference sites after mining (Fig. 3, 1-Yes), the test in step 2 for a different temporal pattern at Har-S is less sensitive (fewer degrees of freedom in the denominator). This condition indicates large natural variation among reference sites over time, and an impact may need to be large to be detect-

Fig. 3. Sequence of questions and statistical tests for asymmetrical analysis of variance (A-ANOVA) to determine if effects of mining at Har-S were significant (Underwood 1993). The question in each box is answered by a standard ANOVA; for example, the *F* value in step 1 equals the mean-square estimate of the interaction among reference sites after mining divided by the mean-square estimate of the residual (T, time; Aft, after mining; Bef, before mining; Ref, reference sites; Sc, scalping at Har-S; EPT, Ephemeroptera, Plecoptera, and Tricoptera; Simp D, Simpson's diversity; Simp E, Simpson's evenness; PC, principal component). Shaded boxes indicate the endpoints of statistical tests for the parameters examined in our study.



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able (Underwood 1993). The most rigorous test for impact is available when the temporal interaction among reference sites is nonsignificant after mining (1-No) and an interaction between Har-S and the reference sites is not detected (2b-No). A detectable impact then produces an interaction in the difference between Har-S and the reference sites before mining compared with after mining (steps 4 and 5).

A short-term interaction between Har-S and reference sites (2a-Yes or 2b-Yes) implies that the temporal trend at Har-S falls outside that found at the reference sites. Accordingly, there must be no change in the interaction from before to after mining among the reference sites (step 3A), and the change in this temporal interaction must be coincident with mining (step 3B). Indeterminate results are produced when variance is large relative to the number of pretreatment sampling episodes. In such cases, we then examined the data graphically, depicted as normal-transformed variables to reflect the scale on which statistical analyses are based, for causal inference.

Juvenile fish

Juvenile fish were collected using a beach seine net (12.5 m × 2 m, 6 mm mesh) to compare the assemblage of species between sites over time. All fish were identified to species, measured (mm), weighed (g), and then promptly released. Each seine haul constituted a sample, and seine hauls were carried out within all habitat units present at each site based on the classification for gravel-bed rivers of Church et al. (2000). This classification recognizes seven habitat types associated with gravel bars: bar head, bar edge, bar tail, eddy pool, open nook, channel nook, and bay. In the gravel reach, these habitats occur at a scale of 10–100 m in perimeter. Habitat-specific sampling effort varied between sites and dates based on habitat unit presence, despite intentions to sample from all habitats on each date. Only bar edge habitat was sampled at all sites on all dates; it is the dominant habitat in the gravel reach and is defined as shallow-sloping (<5°) gravel bar edge oriented parallel to the flow and subject to constant and consistent flow forces of moderate velocity (<50 cm·s^{-1}).

We examined five fish community metrics separately using A-ANOVA at two spatial scales: the bar-scale (seines from all habitat types pooled) and habitat-scale (bar edge seines only). Analyzing data at both spatial scales allowed us to examine to what physical scale the ecological response best corresponded and to accommodate the dilemma that bar-scale analysis pools habitat-specific variability, whereas habitat-scale analysis reduces sample size. The following metrics were included after transformation to meet assumptions of normality and homogeneity of variance: log density (number·10 m^{-2}), % salmonids (arcsine-transformed), species richness, Simpson's diversity, and Simpson's evenness (no transformation).

Because the experimental gravel removal was not anticipated when sampling began, data collection was incomplete for ANOVA requirements at some reference sites. Following Underwood (1997), "dummy values" were substituted for missing data (1% of bar-scale cases, 5% of habitat-scale cases) by using the mean of the other reference sites on that date. The residual degrees of freedom were adjusted to compensate for missing values.

Benthic invertebrates

Three replicate invertebrate samples were collected using a Surber net (500 μm mesh, 0.09 m^2) at each site within habitats with flow (riffle, bar head, bar edge, bar tail). Sampling effort varied between months because habitat availability changed as water levels fluctuated. Samples were field-preserved in 4% formalin and wet-sieved (250 μm mesh) in the laboratory for sorting and identification. No subsampling occurred. Mayflies, stoneflies, and caddisflies were identified to genus; dipterans to either family or subfamily (Chironomidae); beetles and true bugs to family; oligochaetes, leeches, crustaceans, and mites to class; and nematodes to phylum.

We examined six invertebrate community metrics separately using A-ANOVA both at the bar (all habitats pooled) and habitat scale (bar edge only). The following metrics were included after transformation to meet assumptions of normality and homogeneity of variance: log density (number·m^{-2}), % EPT (Ephemeroptera, Plecoptera, and Tricoptera, arcsine-transformed), total and EPT richness, Simpson's diversity, and log Simpson's evenness. We replaced missing data with dummy values following Underwood (1997) for three cases of missing data (1.5% of habitat-scale cases) at reference sites before mining. The residual degrees of freedom were adjusted accordingly.

Densities of common taxonomic families (each representing >1% of invertebrates collected) were compared between sites before and after mining using A-ANOVA for insight into recolonization of a mined gravel bar. Six families met the 1% criterion: Baetidae, Heptageniidae, Ephemerellidae, Capniidae, Chironomidae, and Oligochaeta. Together, they represented 96% of invertebrates collected in our study. Densities were log-transformed prior to analysis to meet assumptions of normality and homogeneity of variance.

Habitat characteristics

Habitat characteristics were measured at all fish sampling sites. Water velocity and depth measurements were taken at nine points within the sampled area using a wading rod and Marsh–McBirney electromagnetic velocity meter. Surface sediment was visually classified for embeddedness and percent representation by major grain-size classes. The slope angle of the bank was calculated as the sine function of maximum sample depth divided by sample width.

Principal components analysis (PCA) was used to summarize total variation in the characteristics of bar edge habitat units and reduce the number of variables to an orthogonal, linear subset representing the dominant physical gradients. These PC axes were then used to examine habitat differences between Har-S and the reference sites before and after mining by considering the relation among all physical factors simultaneously. The following variables were included after transformation to meet assumptions of normality and homogeneity of variance: $\log_{10}$ bank angle, mean depth, maximum depth, mean velocity, maximum velocity, and the arcsine-transformed proportions of cobble, gravel, and sand. A-ANOVA was applied to each of the first three PC axes to determine if the physical characteristics of bar edge units changed as a result of gravel mining.

Sedimentology

Surface sediment samples were collected from the inner bar and water edge of Har-S and Har-R once before (February 2000) and three times after mining (April and September 2000, September 2001). Sampling followed either the Wolman or photographic technique (Church et al. 1987) to measure sediment size and sand coverage. Wolman samples consisted of 400 independently sampled stones and the size-frequency curve was plotted to determine median grain size (D_{50}) and the size of the coarse (D_{95}) sediment present. The photographic method was used only in September 2001 based on a calibration data set from the gravel reach (Rice et al. 2008), from which size percentile metrics were derived.

Single-factor ANOVA was used to compare surface sediment over time between samples collected before (February 2000) and on two dates after gravel mining (September 2000, 2001). Separate analyses were run for inner bar and water edge samples. Three parameters were examined after transformation to meet assumptions of normality and homogeneity of variance: arcsine-transformed proportion of sand and untransformed D_{50} and D_{95}. The critical value was adjusted by the Bonferroni method because multiple significance tests were performed on the same data ($p = 0.05$ divided by four contrasts = 0.0125).

No sampling occurred downstream of the removal area to examine sand deposition as a result of mining. Fine sediment is highly transient in the gravel reach, and considering an average 5.5 million tonnes of sand are transported through the reach each year (McLean et al. 1999), a change in sand content on downstream gravel bars would be undetectable.

Bar topography

Har-S was surveyed in February 2000 and immediately after scalping in March 2000 to determine the volume of gravel removed. The survey was repeated in February 2001, October 2001, and March 2003 to quantify bar-scale sediment recruitment to Har-S after each of three flood events and to measure habitat-scale topographic change. The extent of surveys after mining did not exactly match; thus, we examined the largest area common to all surveys and refer to it as Lower Harrison Bar. This area excluded the outer corner of Har-S but included a larger area of the inner bar to ensure that bar-scale changes in topography were captured.

Volumetric change between each survey of Lower Harrison Bar was estimated using the TOPOGRID (5 m grid spacing) and CUTFILL commands in Arc/Info Workstation (version 8.3, Environmental Systems Research Institute, Redlands, California). Habitat-scale topographic change was examined by creating hypsometric curves to relate bar surface area and elevation based on the TOPOGRID surfaces. Bar elevation was then related to river discharge by regression based on stage readings from a gauge at the mouth of Harrison River and commensurate discharge data at Hope to determine the amount of bar area exposed at high flows. This question was of interest because the lowering of bar surface elevation by scalping may reduce the availability of shallow, nearshore habitat for fish during flooding.

Potential effects of the removal on channel gradient and upstream–downstream changes to sediment supply were not evaluated in this study because they are considered to be negligible. Islam (2008), using the advanced morphodynamic simulation model MIKE21C, calculated that mining as much as 500 000 m^3 of sediment from Harrison Bar would induce a drop in water level, locally, of about 35 mm at the highest probable flood stage. This would affect local river gradient (400 mm·km^{-1}) by less than 10% and have only a small influence on sediment transfer along the river, which would soon be neutralized by renewed deposition on the bar. In comparison, the experimental gravel removal under study was nearly an order of magnitude smaller (69 000 m^3). Water levels and sediment transfer at Harrison Bar are dominated by the hydraulic effect of the sharp channel bend opposite the Harrison River confluence (Fig. 1). Any gravel removal from the bar that does not cause channel realignment is apt to have water level and sedimentation effects that are practically impossible to measure.

Results

Juvenile fish

We report only on bar-scale analyses of fish data because A-ANOVA results were similar at the bar and habitat scales, but the former had higher power to detect an effect when all habitat types were pooled (242 total seines versus 124 seines in bar edge units). A total of 12 094 fish representing 24 species were captured by beach seine at all sites during our study. The number of beach seines differed each month (inset box, Fig. 4) because of varying habitat unit presence; fish sampling could not be conducted at flows > 5700 $m^3 \cdot s^{-1}$.

Fish density varied among sites and seasonally, with highest density between April and September and lowest density in winter months (Fig. 4*a*). In all months after mining except February and August 2001, density at Har-S was equal to or greater than the average density at reference sites (Fig. 4*a*), and no significant effect due to scalping was detected (Table 1).

Species richness was low in winter at all sites compared with spring and summer (Fig. 4*b*). At Har-S, richness was higher than or within the range of values at reference sites in all months except August 1999 before mining and September 2000 and August 2001 after removal (Fig. 4*b*). No effect due to bar scalping could be confirmed, and statistical power was moderate (Table 1).

The proportion of Salmonidae at Har-S was lower than that at reference sites in most months of the study, both before and in the first year after scalping (Fig. 4*c*). The exception was May 2000 when Chinook salmon abundance at Har-S was high. One year after scalping in 2001, the proportion of salmonid species at Har-S was similar to that at reference sites, and no effect due to scalping was detected (Table 1).

Simpson's diversity was lower at Har-S than at reference sites in summer 1999 before scalping and remained lower immediately after scalping in April and May 2000 (Fig. 4*d*). In all periods after August 2000, Har-S samples had diversity similar to that at reference sites, and no effect due to bar scalping was detected (Table 1).

Simpson's evenness was higher at Har-S in August 1999, 2000, and 2001 compared with reference sites, but varied considerably in the intervening periods (Fig. 4*e*). A signifi-

Fig. 4. Average (± standard error) fish metric values at Har-S and reference sites before and after mining (diamonds, Har-S; circles, Car-R; triangles, Fos-R; squares, Har-R). The date that gravel mining commenced is indicated with a vertical broken line. Lines joining symbols connect sampling episodes and are not meant to represent a trend in the intersampling period. The number of beach seines in each month is indicated in the box.

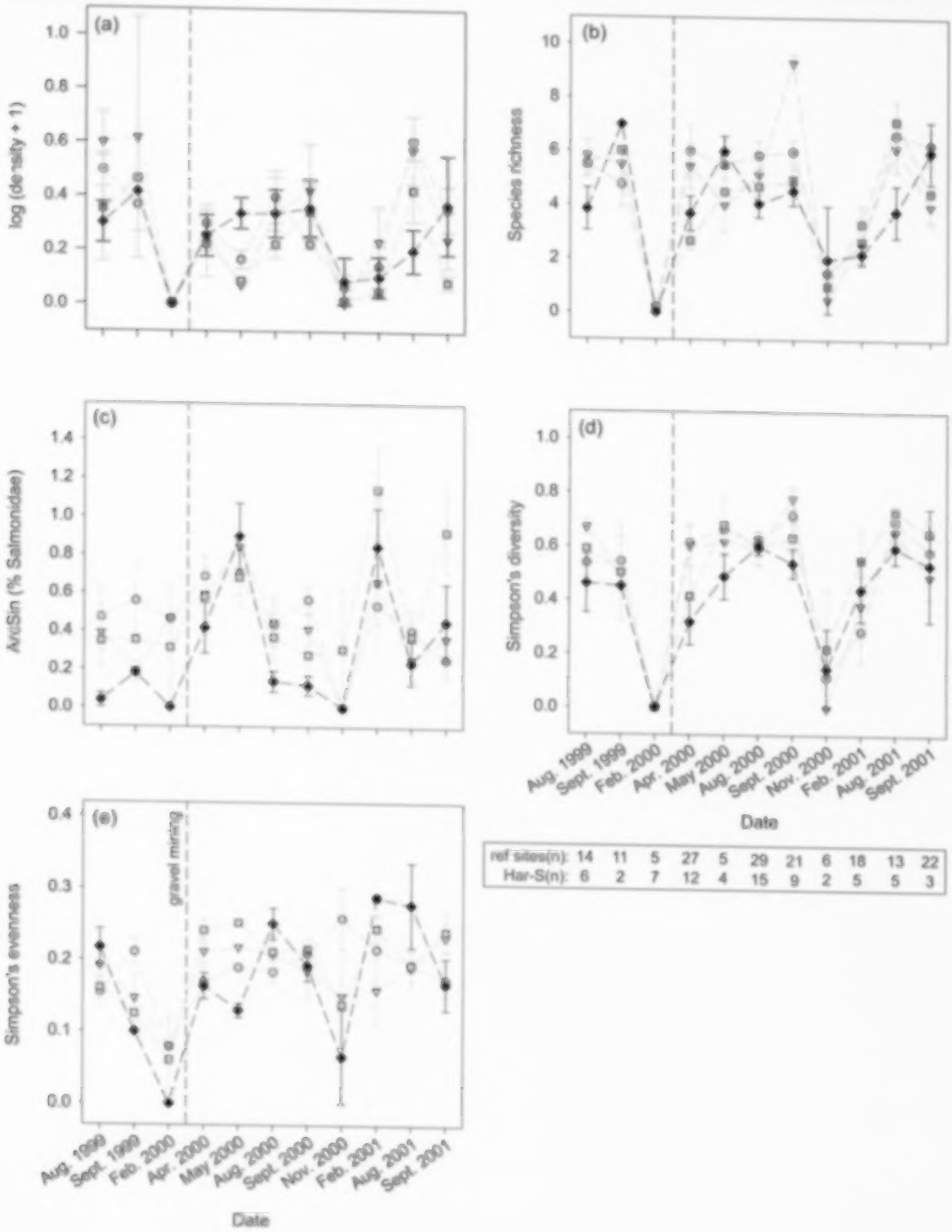


Table 1. Asymmetrical analysis of variance (A-ANOVA) results examining gravel mining impacts at Har-S on fish community metrics (all habitats combined).

Variable	Step 1: Do reference sites have variable short-term trends after mining?	Step 2: Does mining affect short-term temporal trend?	Step 3A: Was change observed at reference sites?	Step 3B: Was timing of change coincident with mining?	Step 4: Do reference sites vary consistently from before to after mining?	Step 5: Does mining affect variation from before to after?	Conclusion	P value	Power
Density	No	No	↑	↑	No	No	No effect detected	0.37	0.18
	No	No	↑	↑	No	No	No effect detected	0.68	0.37
	No	No	↑	↑	No	No	No effect detected	0.27	0.08
	No	No	↑	↑	No	No	No effect detected	0.49	0.26
	No	Yes	Yes	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—

Notes: Refer to Fig. 3 for flowchart showing steps and details of A-ANOVA.

cant temporal interaction between Har-S and reference sites after mining was detected, but the analysis had inadequate power to attribute mining as the cause (Table 1). Graphical examination was not informative in this regard.

Benthic invertebrates

We report only on habitat-scale analyses of invertebrate data because A-ANOVA results were identical at the bar and habitat scales, but the latter had higher power to detect an impact. Invertebrate density varied seasonally at all sites, and February samples contained more than four times the density of animals in August and September (Fig. 5*a*). Year-to-year variability was evident as well. Mean density at Har-S was similar to that at reference sites before mining but lower in May and August 2000 when samples were collected within the removal boundary and from sediment directly disturbed by scalping. A-ANOVA detected a significant temporal interaction among reference sites after mining (Table 2) but lacked statistical power to attribute mining as the cause. The short-term difference detected by A-ANOVA was more likely higher density at Har-S in January 2001 compared with reference sites (Fig. 5). On all dates after August 2000, density at Har-S was higher than the average of reference sites (Fig. 5*a*).

The proportion of EPT was highest in samples from Har-S before scalping, and values remained higher than at reference sites immediately after scalping in April 2000 (Fig. 5*b*). The proportion of EPT at Har-S in all subsequent months was within the range observed at reference sites, and differences between sites were small (Fig. 5*b*). A-ANOVA detected a significant change at Har-S (Table 2), which graphical examination suggested was the positive difference in April 2000.

Both total richness and the number of EPT taxa followed a seasonal cycle similar to density, being highest in winter samples collected between November and February (Figs. 5*c*, 5*d*). Har-S had higher values than the average at reference sites both before and one year after scalping. The lower number of EPT taxa at Har-S in May and August 2000 samples was not statistically significant, and no effect due to scalping was detected for either metric (Table 2).

Simpson's diversity was highest at Har-S prior to scalping but low relative to reference sites in April 2000 when a high proportion of *Ameletus* sp. was collected (Fig. 5*e*). Between May and November 2000, diversity at Har-S was higher than the average of reference sites (Fig. 5*e*) but was lowest in January 2001 when Chironomidae and Baetidae dominated Har-S samples. These differences between Har-S and reference sites were not significant (Table 2).

Simpson's evenness was highest in months immediately after scalping at most sites, including Har-S, and lowest in winter months (Fig. 5*f*). The numerical dominance of Orthocladiinae contributed to low evenness values in February of both years at all sites. A-ANOVA revealed a short-term change at Har-S (Table 2) that likely corresponded with January 2001 samples having notably lower evenness than all reference sites (Fig. 5*f*).

Baetidae abundance at Har-S was approximately equal to or higher than the average at reference sites in all months after mining (Fig. 6*a*). Whereas abundance declined between November 2000 and February 2001 at reference sites, it in-

Fig. 5. Average (± standard error) invertebrate metric values at Har-S and reference sites before and after mining (diamonds, Har-S; circles, Car-R; triangles, Fos-R; squares, Har-R). The date that gravel mining commenced is indicated with a vertical broken line. Lines joining symbols connect successive sampling episodes and are not meant to represent a trend in the intersampling period. EPT indicates Ephemeroptera, Plecoptera, and Tricoptera.

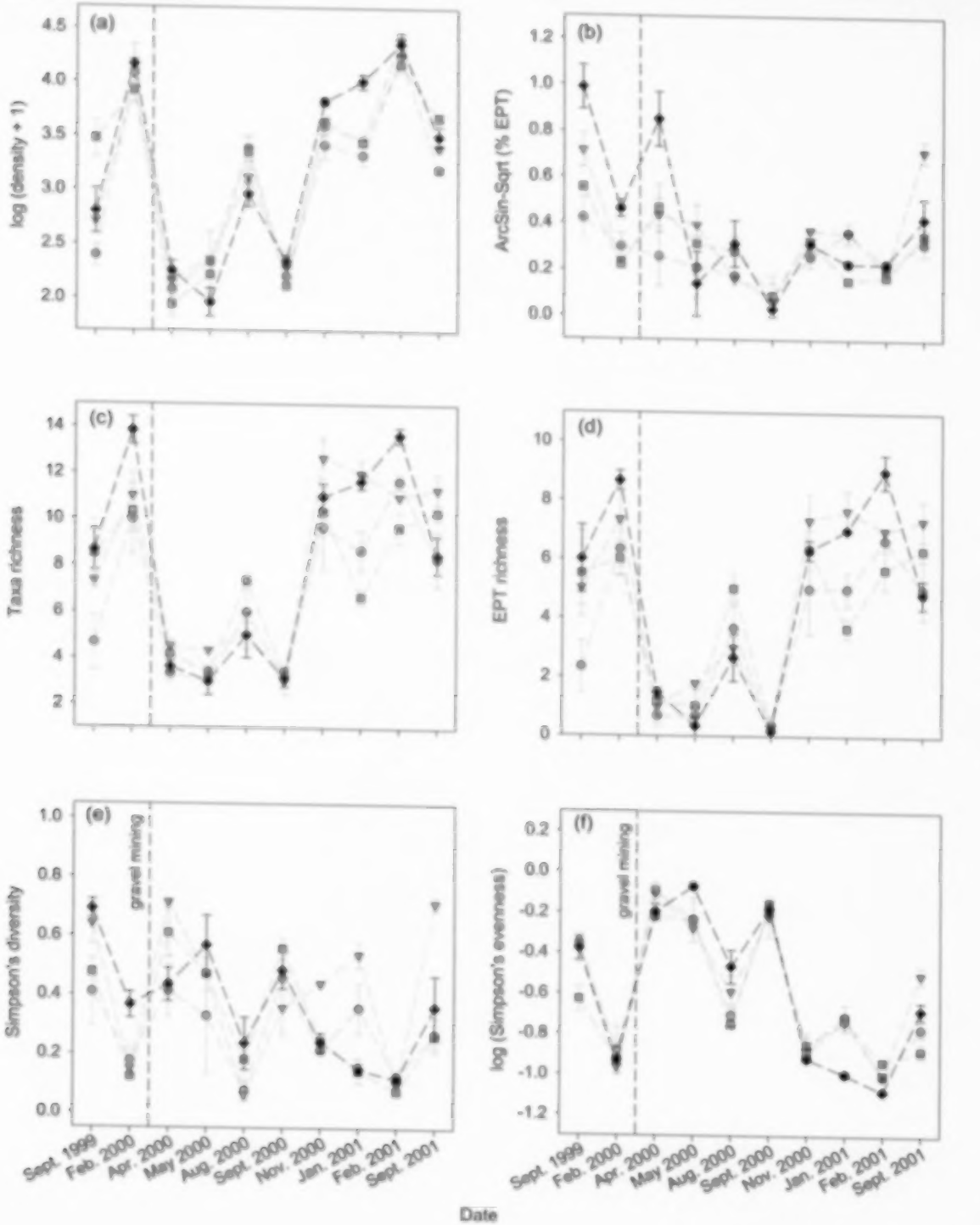


Table 2. Asymmetrical analysis of variance (A-ANOVA) results examining gravel mining impacts at Har-S on invertebrate community metrics (bar edge habitat only).

Variable	Step 1: Do reference sites have variable short-term trends after mining?[1]	Step 2: Does mining affect short-term temporal trend?[2]	Step 3A: Was change observed at reference sites?[3]	Step 3B: Was timing of change coincident with mining?[3]	Step 4: Do reference sites vary consistently from before to after mining?[4]	Step 5: Does mining affect variation from before to after?[5]	Conclusion	*P* value	Power
Density	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—
% EPT[6]	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—
Taxon richness	Yes	No	—	—	—	—	No short-term effect detected	0.39	0.54
EPT richness	Yes	No	—	—	—	—	No short-term effect detected	0.21	0.67
Simpson's diversity	Yes	No	—	—	—	—	No short-term effect detected	0.35	0.62
Simpson's evenness	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—

Notes: Refer to Fig. 3 for flowchart showing steps and details of A-ANOVA.
[6]EPT: Ephemeroptera, Plecoptera, Trichoptera.

creased at Har-S. Notably higher abundance at Har-S compared with reference sites in January 2001 (Fig. 6*a*) likely explains the statistically significant A-ANOVA results (Table 3).

Chironomids (mostly Orthocladiinae) were the most abundant taxon in most samples on all dates. A significant short-term interaction between Har-S and the reference sites is detected (Table 3), but only in January 2001 did abundance at Har-S fall outside, and above, values observed at reference sites (Fig. 6*b*).

Capniidae is the only taxonomic group for which the temporal trend was consistent among reference sites after mining, and no short-term interaction between Har-S and the reference sites was found (Table 3). This allowed for more rigorous impact assessment and no significant effect due to mining was detected. Capniidae abundance was highest in winter months at all sites (Fig. 6*c*).

Patterns of abundance were similar for Heptageniidae and Ephemerellidae families (Figs. 6*d*, 6*e*). Whereas Har-S had higher relative abundance in the summer prior to mining, the families were uncommon at both Har-S and reference sites in spring and summer after removal. Abundance was variable among sites in the winter following mining, but Har-S was generally similar to or higher than the average of reference sites during this period. No significant short-term effect at Har-S was detected for either taxon (Table 3).

Oligochaeta (mostly Naididae and Tubificidae) abundance was the most variable among sites of all families examined (Fig. 6*f*). On most dates after mining, abundance at Har-S was similar to or exceeded values at reference sites, and power to detect an impact was high (Table 3).

Habitat characteristics

Bar edge habitat dominated both Har-S and Har-R prior to mining, and individual units were up to 500 m in length. Increased topographic complexity as a result of flooding in the 3 years after scalping reduced the length of individual bar edge habitat units and increased the variety of habitat types overall.

PCA applied to bar edge habitat characteristics at Har-S and the three reference sites accounted for 90.5% of the total variation in the first three PC axes (Table 4). PC1 explained 44.3% of the variation and represented a hydraulic gradient of increasing water depth, velocity, and bank angle. PC2 and PC3 represented gradients of coarse and fine sediment, respectively. There was a seasonal shift along PC1 at Har-S relative to reference sites from shallow and lower velocity conditions in summer months to deeper and faster-flowing water in winter (Fig. 7). The shift was observed over three summers of sampling, and May 2000 PC1 scores for Har-S fell outside the values for reference sites. A-ANOVA found a significant short-term change in this hydraulic gradient at Har-S (Table 5), and graphical examination supports mining as the cause (Fig. 7). PC2 and PC3 values were similar among sites on all dates before and after scalping.

Sedimentology

Surface sediment texture (D_{50}, D_{95}) prior to mining was similar at Har-S and Har-R (Fig. 8) and became finer from the water edge toward the inner bar. Average sand cover

Fig. 6. Average (± standard error) density of common invertebrate taxa at Har-S and reference sites before and after mining (diamonds, Har-S; circles, Car-R; triangles, Fos-R; squares, Har-R). The date that gravel mining commenced is indicated with a vertical broken line. Lines joining symbols connect sampling episodes and are not meant to represent a trend in the intersampling period.

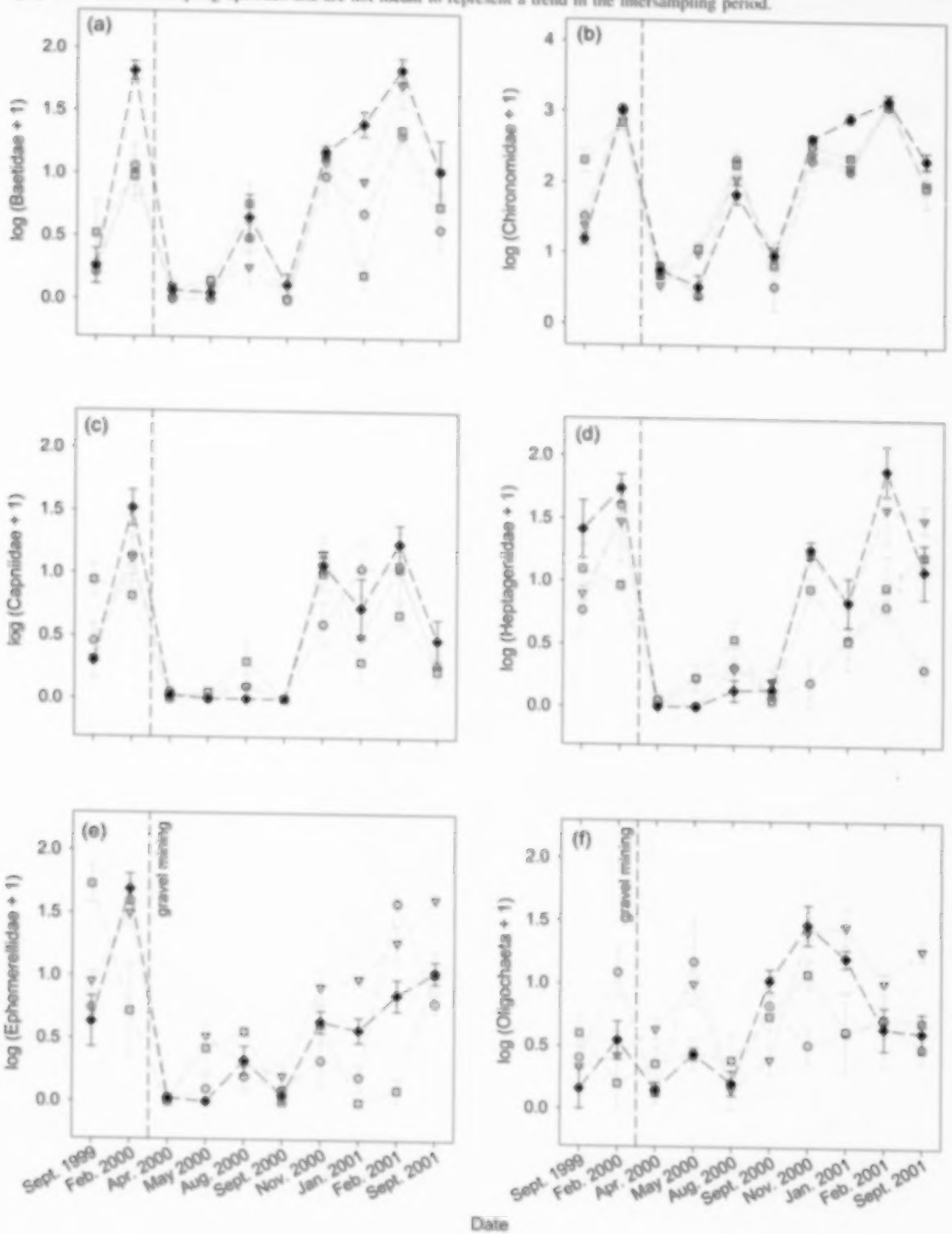


Table 3. Asymmetrical analysis of variance (A-ANOVA) results examining gravel mining impacts at Har-S on common invertebrate family densities.

Variable	Step 1: Do reference sites have variable short-term trends after mining?	Step 2: Does mining affect short-term temporal trend?	Step 3A: Was change observed at reference sites?	Step 3B: Was timing of change coincident with mining?	Step 4: Do reference sites vary in difference from before to after mining?	Step 5: Does mining affect differences from before to after?	Conclusion	*P* value	Power
Baetidae	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	0.03	—
Chironomidae	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—
Capniidae	No	No	↑	↑	No	No	No effect detected	0.71	0.07
Heptageniidae	Yes	No	—	—	—	—	No short-term effect detected	0.29	0.79
Ephemerellidae	Yes	No	—	—	—	—	No short-term effect detected	0.98	0.15
Oligochaeta	Yes	No	—	—	—	—	No short-term effect detected	0.40	0.81

Note: Refer to Fig. 3 for flowchart showing steps and details of A-ANOVA.

Table 4. Factor loadings from principal components analysis (PCA) of bar edge habitat units.

Variable	PC1	PC2	PC3
Cobble	–0.63	0.71*	–0.26
Gravel	0.29	–0.88*	–0.35
Sand	0.55	0.08	0.78*
Bank angle	–0.81*	–0.31	0.44
Average depth	–0.82*	–0.27	0.42
Average velocity	–0.73*	–0.26	–0.27
Eigenvalue	2.66	1.53	1.24
% variation explained	44.3	25.6	20.6

*Correlated with PC axis.

was relatively high at both Har-S (11%) and Har-R (17%) but locally variable. Immediately after scalping, Har-S had a higher proportion of sand, average D_{95} decreased from 66 to 39 mm, and average D_{50} decreased from 25 to 13 mm. After two below-average floods, the D_{50} at Har-S in September 2001 was nearly identical to the size before removal both along the water edge and inner bar. Average D_{95} was less than that before scalping at the water edge, but a reduction in size over this period was observed at Har-R as well. Sand content in both areas was lower in September 2001 than before scalping.

Single-factor ANOVA comparing values at Har-S over time showed that the inner bar had significantly higher sand content before scalping compared with both sampling dates after scalping (Table 6). The gravel fractions were similar in size before and after scalping.

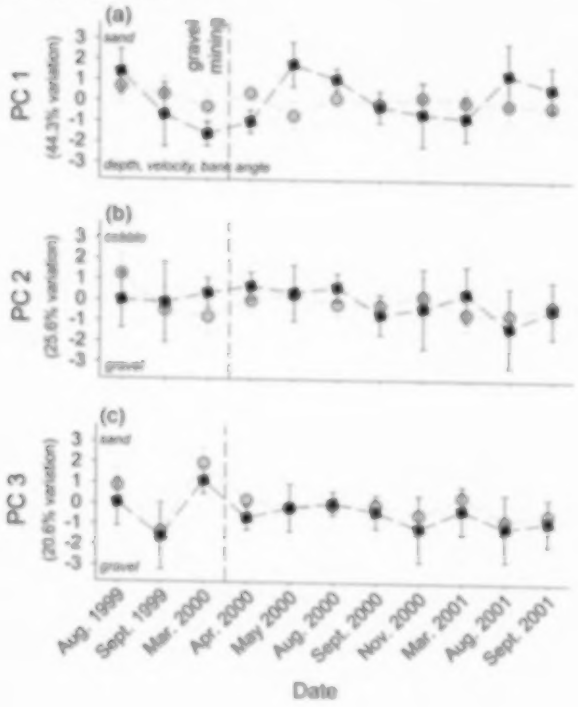
Bar topography

The topography of Lower Harrison Bar (the area commonly surveyed in all years) was simple prior to mining (Fig. 9*a*), and average and maximum surface elevations were 8.4 and 11.6 m, respectively (Table 7). Scalping removed 69 070 m^3 of sediment from within Har-S (62 232 m^3 from Lower Harrison Bar, the difference due to exclusion of the outer corner of Har-S) and left a low-gradient slope running from the inner bar to the water edge (Fig. 9*b*). The maximum vertical depth of extraction was nearly 2 m, and average surface elevation was reduced by approximately 1 m.

The modest 2000 and 2001 floods resulted in further net losses of 6635 and 1676 m^3 from Lower Harrison Bar, respectively (Table 8). There was, however, sediment exchange (erosion and deposition) over the bar in both years that filled the major area that had been reduced to <8 m elevation (Fig. 10) and increased topographic complexity at the habitat scale (Figs. 9*c* and 9*d*). A summer channel developed across the lower corner of the bar during flooding in 2000 (Fig. 9*c*) that had irregular geometry, high habitat diversity, and conveyed flow through October 2000 (discharge > 1500 $m^3 \cdot s^{-1}$). Sediment deposition by the 2001 flood cut off flow into the channel after September 2001 (discharge > 1800 $m^3 \cdot s^{-1}$), and in 2002 the channel carried flow through August (discharge > 2000 $m^3 \cdot s^{-1}$).

The large 2002 flood produced the most notable topographic change, depositing 27 630 m^3 of sediment. Comparing February 2000 and March 2003 surveys, there remained

Fig. 7. Mean factor scores (±95% confidence interval) for Har-S and reference sites derived from principal component analysis (PCA) of bar edge habitat characteristics (squares, Har-S; circles, reference sites). Variables most highly correlated with each axis are indicated in each panel. The date that gravel mining commenced is indicated with a vertical broken line. Lines joining symbols connect sampling episodes and are not meant to represent a trend in the intersampling period.



a net loss of 42 913 m^3 over Lower Harrison Bar (Table 8). With 62 232 m^3 removed from this same area, 31% was replenished by the 2002 flood to restore average bar elevation to within 10 cm of the surface before scalping (Table 7). Comparing these two surveys also reveals the extent to which topographic complexity increased across Lower Harrison Bar from before to after mining (Figs. 9*a*, 9*e*). Based on the relation between discharge (Q) and water surface elevation ($z = 0.0005Q + 6.55$, $r^2 = 0.98$), Lower Harrison Bar would have been submerged at a discharge of 10 100 $m^3 \cdot s^{-1}$ prior to mining compared with 10 900 $m^3 \cdot s^{-1}$ after the 2002 flood (maximum bar elevations of 11.6 and 12.0 m, respectively, Table 7).

Bar scalping resulted in a major shift in the elevation profile of Lower Harrison Bar that was partially reversed by flooding (Fig. 10). For example, the proportion of bar with >9 m elevation declined from 24% before scalping to 0% immediately after scalping and 18% after three flood events (Table 7). Hence, there remained a net 25% loss of bar area with >9 m elevation after the 2002 flood. Despite no long-term reduction in maximum surface elevation, this remaining deficit in high bar area, which provides shallow-water habitat for fish during flood, was notable (Table 7).

Table 8. Asymmetrical analysis of variance (A-ANOVA) results examining gravel mining impacts on the physical characteristics of bar edge habitat units at Har-S.

Variable	Step 1: Do reference sites have variable short-term trends after mining?	Step 2: Does mining affect short-term temporal trend?	Step 3A: Was change observed at reference sites?	Step 3B: Was timing of change coincident with mining?	Step 4: Do reference sites vary consistently from before to after mining?	Step 5: Does mining affect variation from before to after?	Conclusion	*p* value	Power
Hydraulic gradient (PC1)	No	Yes	No	No	—	—	Inadequate degrees of freedom to confirm an effect due to mining	<0.001	—
Coarse sediment gradient (PC2)	No	No	↑	↑	No	No	No effect detected	0.72	0.004
Fine sediment gradient (PC3)	No	No	↑	↑	No	No	No effect detected	0.48	0.04

Note: Refer to Fig. 3 for flowchart showing steps and details of A-ANOVA.

Fig. 8. Surface sediment characteristics (mean ± standard error) in the scalped (black symbols) and reference (grey symbols) areas of Harrison Bar before and after gravel mining along the water edge (circles) and inner bar (squares). The date that gravel mining commenced is indicated with a vertical broken line. Lines joining symbols connect sampling episodes and are not meant to represent a trend in the intersampling period.

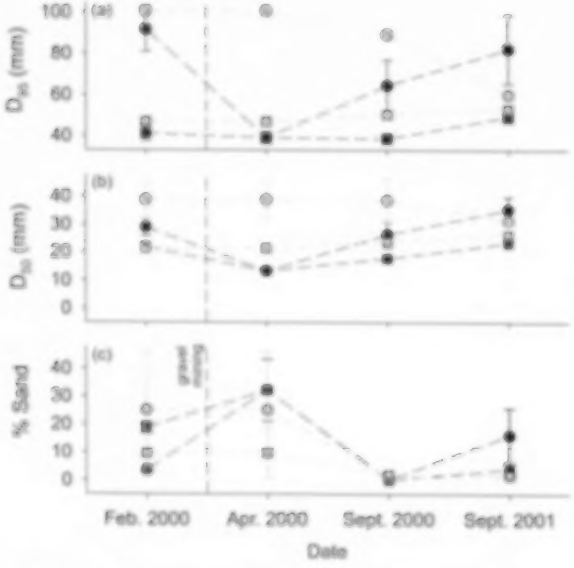


Table 6. Analysis of variance (ANOVA) results comparing surface sediment samples before and after gravel mining.

Parameter	df (residual)	MS (residual)	F	p
Water edge				
% sand	2 (3)	0.04 (0.05)	0.83	0.52
D_{50}	2 (3)	43.3 (57.8)	0.75	0.54
D_{95}	2 (3)	384.3 (724.9)	0.53	0.64
Inner bar				
% sand	2 (3)	0.1 (0.003)	33.31	0.009*
D_{50}	2 (3)	19.3 (11.5)	1.67	0.32
D_{95}	2 (3)	33.5 (40.2)	0.83	0.52

Note: D_{50}, median sediment size; D_{95}, coarse sediment size; df, degrees of freedom; MS, mean square.

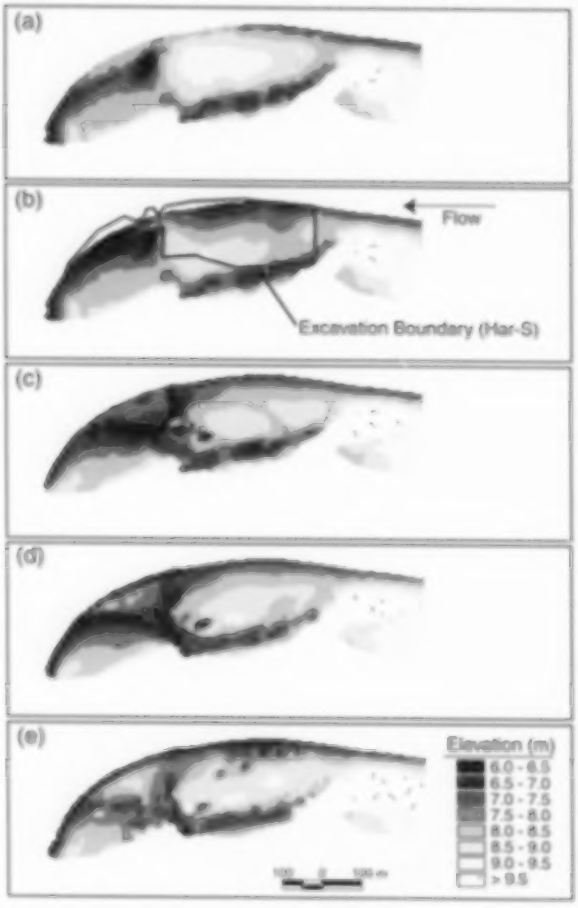
*$p < 0.0125$, corrected by Bonferroni's method for multiple contrasts.

Discussion

Physical changes

Immediate physical changes to Harrison Bar as a result of gravel mining in March 2000 were substantial. Scalping removed 69 070 m^3 of sediment, and the pre-existing coarse and stable bar surface was replaced by an evenly graded area of loose gravel and sand. However, the dramatic change in sediment texture was short-lived, as flooding in 2000 transformed the loose and sandy substrate into a moderately coarse surface with negligible sand cover. Reduced sand cover over both the scalped and reference areas of Harrison Bar after flooding indicates that sand entrainment across the entire bar surface was high. These observations are consistent with the transient occurrence of sand in the gravel reach, and considering the 5.5 million tonnes of sand transported through the reach each year on average (McLean et al. 1999), sand transport from Har-S is believed not to have had a detectable or lasting impact on downstream habitats. Flooding in 2001 produced additional surface coarsening within the removal area, although the coarsest fraction (D_{95}) along the water edge remained smaller than that prior to mining.

Fig. 9. The topography of Lower Harrison Bar before and on four dates after gravel mining: (*a*) Feb. 2000, (*b*) Mar. 2000, (*c*) Feb. 2001, (*d*) Oct. 2001, (*e*) Mar. 2003.



Sedimentary changes at Har-S as a result of flooding occurred concurrently with volumetric and topographic changes. Modest flooding in 2000 and 2001 yielded no volumetric replenishment but resulted in minor rebuilding of high bar habitat and increased topographic complexity due to sediment exchange across the bar surface. In the gravel reach, topographic complexity begets habitat diversity, and the increased topographic complexity over Har-S after flooding in 2000 and 2001 resulted in higher habitat diversity at the site overall. Therefore some habitat-scale recov-

Table 7. Surface elevation characteristics of Lower Harrison Bar before and on multiple dates after gravel mining (area: 247 825 m^2).

Date	Period	Elevation (m)			Elevation > 8 m*		Elevation > 9 m*	
		Mean	Max.	Min.	Area (m^2)	%	Area (m^2)	%
Feb. 2000	Before scalp	8.4	11.6	5.7	175 725	71	58 925	24
Mar. 2000†	After scalp	7.2	9.7	5.5	9 775	4	<1 000	<1
Feb. 2001	After flood 1	8.1	10.9	6.1	135 900	55	35 300	14
Oct. 2001	After flood 2	8.1	11.9	5.8	136 950	55	38 250	15
Mar. 2003	After flood 3	8.3	12.0	5.6	141 250	57	44 750	18

Note: Lower Harrison Bar represents the area commonly included in all surveys after mining.

*The areas of bars with elevations >8 m and >9 m would remain exposed at discharges of 2960 and 4960 $m^3 \cdot s^{-1}$, respectively.

†Values for March 2000 are derived from within Har-S removal area rather than from Lower Harrison Bar.

Table 8. Volumetric (m^3, bulk volume) comparisons between surveys of Lower Harrison Bar.

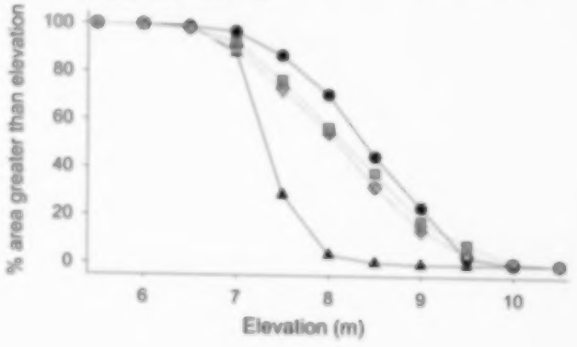
Survey contrasts	Erosion	Deposition	Net change
Feb. 2000 vs. Mar. 2000 (mining)	–63 881	+1 648*	–62 232†
Mar. 2000 vs. Feb. 2001 (2000 flood)	–47 476	+40 840	–6 635
Feb. 2001 vs. Oct. 2001 (2001 flood)	–28 414	+26 737	–1 676
Oct. 2001 vs. Mar. 2003 (2002 flood)	–23 348	+50 978	+27 630
Feb. 2000 vs. Mar. 2003	–81 317	+38 400	–42 913

Note: Lower Harrison Bar represents the area commonly included in all surveys after mining and excludes the downstream, outer corner of Har-S (see Fig. 9b).

*Minor deposition due to a sediment berm left by the contractor at the downstream end of Har-S.

†Discrepancy with reported volume of 69 070 m^3 due to exclusion of the downstream corner of Har-S in the survey comparison.

Fig. 10. Area–elevation relation of Lower Harrison Bar based on topographic surface modeling (circles, Feb. 2000; upright black triangles, Mar. 2000; diamonds, Feb. 2001; reverse grey triangles, Oct. 2001; squares, Mar. 2003).



ery occurred in these two low-flood years despite the fact that bar-scale recovery in sediment volume did not. In 2002, above-average flooding replenished 31% of the scalped volume and restored average bar surface elevation to within 10 cm of the average before mining. Maximum bar elevation exceeded the level before scalping by 35 cm. These findings demonstrate that vertical bar growth can proceed after gravel mining so long as an upstream supply of sediment is recruited to the site during high flow events.

Despite modest sediment replenishment and vertical bar growth over Lower Harrison Bar, the proportion of area at elevations > 9 m after the 2002 flood remained 25% smaller than that before mining. The loss of high bar habitat was most significant for flows > 5000 $m^3 \cdot s^{-1}$, generally from May through August, when these areas represent nearshore rearing habitat for juvenile fish. Habitat availability naturally decreases in summer months as flooding inundates gravel bars and low-lying islands. The reduction in high elevation bar area as a consequence of mining would have further reduced available rearing habitat during a period when it is already limiting for fish in the gravel reach (Perkins 2007). Only by comparing the area–elevation relation and then relating it to the specific range of flows over which fish might be affected was this impact to fish habitat identified.

The extent to which the effect of this high-bar habitat loss transmitted to fish populations remains uncertain. Fish species may have alternate strategies to cope with seasonal flooding in Fraser River, especially given the river's highly predictable hydrograph. Several studies of northern floodplain rivers have documented lateral shifts in the distribution of fish during high flow events (Sommer et al. 2001; King et al. 2003; Schwartz and Herricks 2005), with side channels becoming increasingly important. Flood predictability and duration are believed to be important factors influencing seasonal side channel use for spawning and rearing and as refugia from flooding (Galat et al. 1998; King et al. 2003). In Fraser River side channels, gillnet and minnow trap sampling during spring flooding consistently yielded high catch rates (L.L. Rempel, unpublished data), but comparable data during flooding from main channel and bar top habitats are not available.

The overall physical transformation of Har-S by flooding highlights the critical role of sediment transport for maintaining fish habitat in the gravel reach and in gravel-bed rivers in general. Even in the absence of gravel mining, bars undergo changes in sediment texture, topography, and volume on an annual basis that create habitat at a local scale and cause lateral instability at the bar scale (Rice et al. 2008). Sediment transport also is important for maintaining

high-quality habitat by episodically reworking and cleaning the substrate (Milhous 1982; Resh et al. 1988; Kondolf and Wilcock 1996). Such fluvial processes and the relatively dynamic presence of habitat units characterize the natural state of the Fraser River gravel reach to which local populations of aquatic organisms are likely accustomed and which may habituate them to modest imposed disturbances.

Response by aquatic organisms

More than 12 000 fish representing 24 species were captured by beach seine during this study. Chinook salmon were numerically dominant and most commonly found in the main channel occupying bar edge habitat as well as eddy pools and channel nooks. The extensive use of the Fraser River by juvenile Chinook salmon has been documented previously (Brown et al. 1989; Levings and Lauzier 1991), but its dominant abundance year round in the gravel reach was a novel finding. Large numbers of juvenile Chinook were collected in the former removal area of Harrison Bar soon after mining in May 2000, but Chinook density and percent Salmonidae were lower at Har-S in most subsequent months.

Fish density, along with several other metrics characterizing the fish community, showed no detectable impact at Har-S as a result of mining over the range of flows sampled. The statistical analyses were relatively sensitive because reference sites varied in a consistent manner over time. Only Simpson's evenness changed significantly at Har-S relative to reference sites, but high variance among all sites over time prevented the analysis from confirming that the change was coincident with scalping. More generally, high spatial and temporal variance reduced statistical power overall and confounded our attempt to detect the effect of gravel mining on the fish community at Harrison Bar. A replicated study design, wherein gravel mining occurred at multiple sites, would have been more powerful and possibly generated more definitive results. However, this design was not possible given regulatory restrictions and the moratorium on gravel mining in place.

Benthic invertebrates have been used to examine the ecological response to habitat disturbance in numerous previous studies (e.g., Gurtz and Wallace 1984; McCabe and Gotelli 2000). In our study, benthic invertebrates recolonized Har-S immediately after mining as water inundated the site with the onset of flooding. Samples collected in April 2000 had above average density and included a high proportion of mayfly, stonefly, and caddisfly nymphs. Several of these taxa (e.g., *Ameletus* sp.) are highly mobile and have good swimming ability (Mackay 1992). These behavioral tactics are practical for survival in the gravel reach, where the water edge shifts laterally across the surface of gravel bars during flooding. The rapid colonization of Har-S is consistent with previous work in the gravel reach by Rempel et al. (1999), which showed invertebrates migrate laterally across undisturbed gravel bars with the rise and fall of water levels. Just as *Ameletus* sp. was found almost exclusively in April 2000 samples in this study, it was collected by Rempel et al. (1999) only in April.

In two subsequent sampling episodes after April 2000, invertebrate density at Har-S was lower than that at reference sites. Taxon and EPT richness were lower at Har-S during this period as well, though differences were not statistically significant. These May and August 2000 samples were collected from within the scalped boundary, and it was during this period of high discharge that the bar surface underwent the most dramatic change in sediment texture. The rate of sediment transport across Lower Harrison Bar was probably higher because of the loose material left by scalping (US ACE 1982), and these conditions may have deterred settlement by some taxa or crushed them. But just as sediment texture generally recovered by September 2000, invertebrate samples collected from Har-S in September 2000 and all months thereafter had higher density than the average of all reference sites.

Taxon richness, the number of EPT taxa, and Simpson's diversity each showed variable trends after mining at the reference sites, and no short-term effect at Har-S was detected. Statistical power was relatively high for these analyses despite temporal variability among reference sites. Metrics for which a short-term change was detected (density, % EPT, Simpson's evenness) had more consistent, though still high variance among reference sites after mining relative to those metrics showing no detectable impact. High variance generally characterizes the natural condition for Fraser River, and Underwood (1993) asserts that a significant temporal interaction among reference sites after treatment (i.e., mining) indicates that an impact would have to be large to be ecologically important. Otherwise, it falls within the natural range of variability encountered by populations and therefore should be within the community's capacity to recover.

The fact that virtually all community metrics and taxon-specific densities at Har-S were similar to or higher than those at reference sites after August 2000 implies that the scale of disturbance by a single gravel removal of modest size at Harrison Bar was within the system's capacity to recover. Moreover, aquatic organisms appeared most sensitive to habitat-scale effects of mining because community and taxon-specific metrics recovered soon after the first flood, coincident with the recovery of sediment texture and topographic variability. These habitat-scale physical changes persisted only into the first flood after mining, whereas bar-scale changes in volume and overall topographic distribution remained through at least three freshets. The statistical appearance that fish metrics were best evaluated at the bar-scale is an operational sampling issue related to high variance, which does not upset this conclusion. Our hypothesis that the ecological response to mining is mediated by annual flooding is therefore supported.

This finding, however, can provide a basis for ecologically sound management of gravel mining only up to a point. The experimental removal at Harrison Bar was situated in a reach of persistent sediment aggradation, and the removal volume constituted only a small fraction (approximately 30%) of mean annual sediment recruitment to the gravel reach of Fraser River. We recommend that an essential element of any river gravel management plan is for removal volumes to remain modest in comparison with natural rates of sediment recruitment to ensure that sediment transport processes remain intact. Larger removals may require years of sediment recruitment for recovery, with the potential loss of important bar-scale habitat, particularly

high-bar rearing habitat, for periods exceeding the generation time of most aquatic organisms. The removal of 30% of the average annual recruitment rate was coincidentally similar to Kondolf et al.'s (2002) recommendation for limiting removals to 50% of the annual rate of sediment recruitment to reduce impacts on salmonid spawning habitat in Washington rivers. Implementing this recommendation requires knowledge of basin-scale processes of sediment recruitment and storage and reach-scale patterns of sediment erosion and deposition. These factors influence the system's response to disturbance and capacity for recovery and should be regarded as fundamental factors in the maintenance and management of fish habitat.

Sampling strategy and statistical power

Statistical power is a well-established concept being applied increasingly in studies when results have important implications for resource management (Peterman 1990). Power analysis is particularly useful when planning a study to determine the necessary sampling effort for a desired level of power or to solve for the minimum detectable effect size based on a predetermined sampling effort (Osenberg et al. 1994). In both applications, knowledge of the system's natural variance is required. Variance estimates from our study are useful in this regard for designing future habitat and fishery monitoring programs in the gravel reach of Fraser River.

Determining the most effective sampling strategy (i.e., that returns the most discriminating results without excessive effort) will ultimately depend on the temporal and spatial structure of variance in the data. Large variability in time and space characterized our data, and four exploratory analyses were carried out to evaluate how changes to our sampling strategy might have improved statistical power. The invertebrate metric taxon richness was chosen, and analyses consisted of (*i*) simulating a larger effect size in April 2000 by greatly reducing taxon richness at Har-S; (*ii*) adding a fourth reference site to the analysis (Calamity Bar was included in the sampling program but data were excluded from primary analyses because it is downstream of Har-S); and (*iii*) eliminating several after-mining sampling episodes. The fourth simulation used a hypothetical data set of Underwood (1993) in which we randomly eliminated two of four before-impact sampling episodes to evaluate if the ability of A-ANOVA to detect change was compromised by our unequal sampling episodes before and after mining.

Collectively, these simulations indicated that when spatial variability (i.e., bar to bar) is high, the addition of another reference site does not improve resolution greatly. An additional reference site will, however, improve power when the effect size is very large. Also, the addition of one or two sampling episodes may not greatly increase resolution when there is high temporal variability. In such cases, it is more effective to increase sample replication at all sites to improve as much as possible the estimates of mean values, thereby improving the ability of the analysis to discriminate among them. Lastly, statistical power is virtually unaffected by an unbalanced sampling design compared with the effect of variance in the data set. Hence, our study was not fatally weakened by its unbalanced design. A greater number of sampling episodes prior to mining would have improved estimates of the natural variance, thereby increasing statistical power to detect an impact. Of course, such insights can only be drawn once knowledge of the system's natural variance has been gained. Because a replicated and balanced design is often not realistic for real world, anthropogenic treatments, Underwood's (1992, 1994, 1997) A-ANOVA presents an alternative statistical design for such cases. Our chosen analysis was as rigorous as the data allowed.

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Spatial and temporal impact of pingers on porpoises

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Abstract: Bycatches are considered the most serious threat to harbour porpoises (*Phocoena phocoena*) and other small cetaceans worldwide. Pingers are used to reduce bycatch levels, but may also deter porpoises from critical habitats. We investigated the spatial and temporal responses of porpoises to simulated bottom-set nets equipped with periodically operating Dukane NetMark 1000 pingers. Echolocation rates were monitored by porpoise click train detectors (PODs) placed at and around the nets, and a shore-based observation team recorded surfacing positions and movements. Pinger sound significantly reduced the median echolocation encounter rate by 50%–100% at PODs placed up to 500 m and reduced the sighting rate up to 375 m from the simulated net. The average distance of approach increased by 300 m. When pingers were silent after being active for 24 h 50 min, the return time of porpoises was 6 h, in comparison with 2.5 h after pingers had been silent. During the study period of approximately 50 days, habituation was detectable at two of nine PODs. The results indicate that pingers affect porpoises at greater distances than previously observed. This confirms that pingers are an effective bycatch mitigation measure, but alternative solutions should be applied in ecologically important habitats and migration routes. An example is given from the Baltic region.

Résumé : On considère, qu'à l'échelle globale, les captures accessoires représentent la menace la plus sérieuse aux marsouins communs (*Phocoena phocoena*) et aux autres petits cétacés. Des émetteurs acoustiques sont utilisés pour réduire les captures accessoires, mais ils peuvent aussi éloigner les marsouins de leurs habitats essentiels. Nous avons étudié les réactions spatiales et temporelles de marsouins à des filets simulés fixés au fond et équipés d'émetteurs acoustiques Dukane NetMark 1000 à fonctionnement périodique. Les taux d'écholocation ont pu être suivis à l'aide de détecteurs des séries de claquements de marsouins (PODs) placés sur les filets ou dans les environs et une équipe d'observateurs sur la rive a noté les positions de remontée en surface et les déplacements des marsouins. Le son des émetteurs acoustiques a réduit significativement le taux médian de détections d'écholocation de 50–100% dans les PODs situés jusqu'à 500 m du filet simulé et le taux d'observations en surface jusqu'à 375 m. La distance d'approche moyenne a augmenté de 300 m. Lorsque les émetteurs acoustiques sont silencieux après une période d'activité de 24 h 50 min, le temps de retour des marsouins est de 6 h, alors qu'il est de 2.5 h lorsque les détecteurs restent silencieux. Durant la période d'étude d'environ 50 jours, il a été possible de détecter de l'habituation à deux des neuf PODs. Nos résultats indiquent que les émetteurs acoustiques affectent les marsouins à des distances plus grandes que celles observées précédemment. Cela confirme que les émetteurs sont un moyen efficace de mitigation des captures accessoires, mais il faudrait utiliser des solutions de rechange dans les habitats d'importance écologique et les routes de migration. Nous donnons un exemple provenant de la région de la Baltique.

[Traduit par la Rédaction]

Introduction

Incidental mortalities in gillnet fisheries are considered the most serious threat to dolphins and porpoises worldwide (Dawson 1994; Kraus et al. 1997). The harbour porpoise (*Phocoena phocoena*) is subjected to bycatches throughout its distribution range in the Northern Hemisphere (Jefferson and Curry 1994; Perrin et al. 1994). Several studies have concluded that documented bycatch levels may not be sustainable, e.g., in the Bay of Fundy – Gulf of Maine (Trippel et al. 1996); the Celtic Sea (Tregenza et al. 1997); the central North Sea (Vinther 1999); and the Skagerrak, Kattegat, and Baltic seas (Berggren et al. 2002). The issue is of particular concern for the populations in the Baltic Sea (Berggren et al. 2002; Koschinski 2002) and in the Black Sea – Sea of Azov (Birkun et al. 1999).

Large-scale field experiments in commercial fisheries have demonstrated that acoustic deterrent devices (pingers) substantially reduce the bycatch levels of harbour porpoises in bottom-set gill nets (Kraus et al. 1997; Trippel et al. 1999) and several other marine mammal species in drift gill nets (Barlow and Cameron 2003). To mitigate bycatch of harbour porpoises, pingers are now mandatory in selected combinations of gillnet fisheries, areas, and periods in EU waters (European Commission 2004) and in the Gulf of Maine (National Oceanic and Atmospheric Administration (NOAA) and National Marine Fisheries Service (NMFS) 1998). Pingers are also compulsory in the swordfish–shark drift gillnet fishery in California and Oregon to mitigate bycatches of several species of marine mammals (NOAA and

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NMFS 1997). Although the regulations can give technical specifications for the pingers to be used, there is no uniform standard for the sound characteristics of a pinger, and many questions remain on how various sounds affect the ecology of marine mammals, fish, and other organisms.

The behavioural response of captive harbour porpoises varies with the frequency characteristics of the pinger sounds (Kastelein et al. 1995, 1997, 2001). In the wild, the sound of a pinger has been observed to displace porpoises at the surface (Koschinski and Culik 1997; Cox et al. 2001; Culik et al. 2001) and reduce their echolocation activity at the pinger position (Cox et al. 2001; Culik et al. 2001). Several scientific issues regarding the widespread use of pingers have been raised by Dawson et al. (1998). One of these issues was if long-term exposure will make porpoises habituated to the sound, and pingers thereby lose their effectiveness. While habituation has been detected within a few days of constant pinger operation in one study (Cox et al. 2001), others have not observed such an effect (Koschinski and Culik 1997; Culik et al. 2001). Concern has also been raised about pingers forcing porpoises into suboptimal areas (Culik et al. 2001) and that bodies of water may be cut off if pingers are used in coastal areas with limited access (Carlström et al. 2002).

In the present study, harbour porpoise underwater echolocation activity and surfacing positions were recorded at and around simulated bottom-set gill nets equipped with pingers. The aims were to assess the spatial and short-term temporal impact of pinger sound on porpoise distribution using acoustic and visual methods. Specifically, the objectives of the spatial analyses were to determine at what distances pinger sound affects (*a*) porpoise echolocation encounter rate, (*b*) initial sighting rate, and (*c*) group size. We also investigated how the sound of pingers affected (*d*) the distance between porpoise tracks and the simulated net. The objective of the short-term temporal analyses was to investigate (*e*) the return time of porpoises after pingers had been switched off. Pingers were only active while data were collected for different analyses. The aim of this design was to minimize the risk of harbour porpoises habituating to the pinger sound during the course of the experiment. Consequently, the obtained data were also tested for (*f*) habituation.

Materials and methods

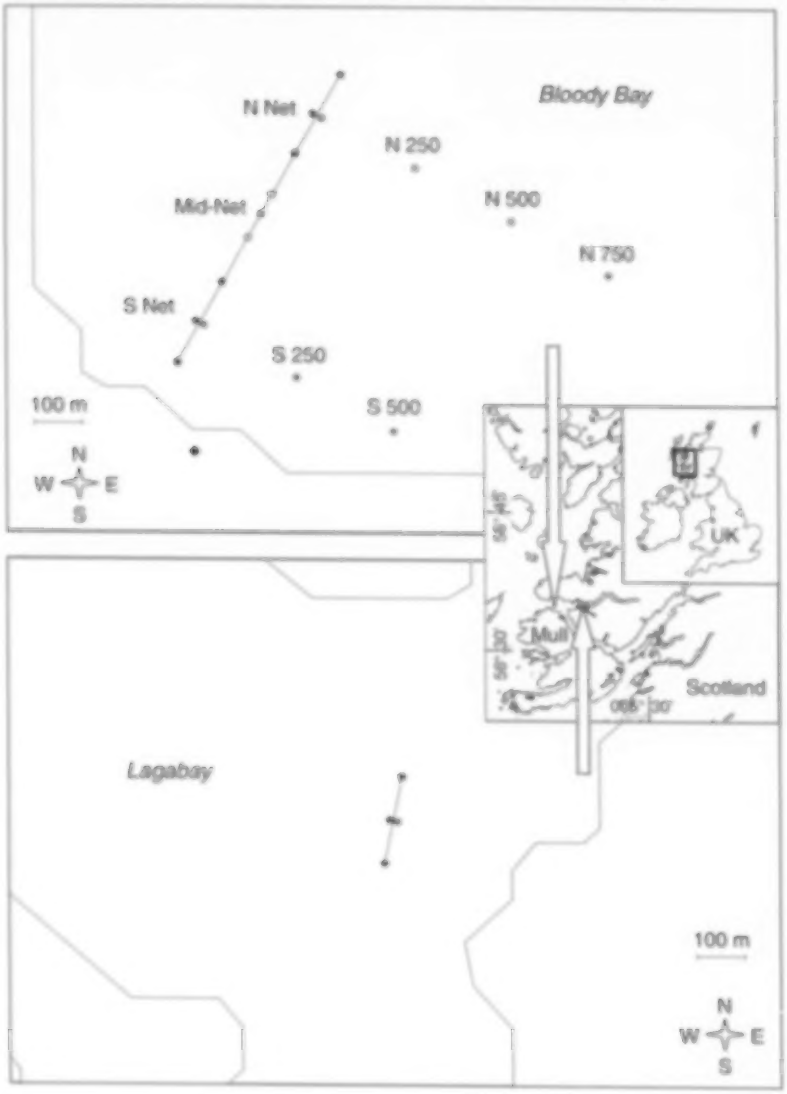
The experiment was carried out at two nearby locations in the waters off West Scotland, UK: Bloody Bay and Lagabay (Fig. 1). In Bloody Bay, both visual and acoustic data were collected from 26 April to 15 June 2001. In Lagabay, only acoustic data were collected from 20 June to 7 August 2001. At both locations, Chelonia T-POD V1 porpoise detectors (PODs) (Chelonia Ltd., www.chelonia.co.uk) and Dukane NetMark 1000 pingers (Dukane Corp., Seacom Division, Dukane Drive, Illinois, USA) were used. The PODs scanned for porpoise clicks at 130 kHz. Series of click signals that were classified as harbour porpoise echolocation click trains by the POD software's detection algorithm were included in the analyses (Chelonia T-POD programme version 22 November 2002). The radial detection probability of the T-POD V1 has been measured using the detection algorithm that accepts all click trains, i.e., trains originating from any source and not only those that are identified as harbour porpoise click trains. With this algorithm, the modelled radial detection function shows that the POD detects close to all porpoises present at the POD position, 50% of all porpoises approximately 60 m from the POD, and 5% of all porpoises approximately 120 m from the POD (Tougaard et al. 2006). Details on how the POD detects and logs signal information are given by Carlström (2005). The Dukane NetMark 1000 pingers have been measured to produce a 0.3 s pulse at 10–12 kHz every 4 s at a level of 133–145 dB (re 1 µPa at 1 m) (Trippel et al. 1999).

In Bloody Bay, a simulated net consisting of a 700 m long lead line carrying eight pingers and three PODs was set on the flat muddy–sandy bottom at 42 m depth. The pingers were spaced by 100 m and the PODs by 250 m. The position of the central pinger was 56°38.92′N, 6°06.00′W. An additional five PODs were deployed at distances of 250, 500, and 750 m from the net. In Lagabay, a 200 m long lead line carrying three pingers and one POD was set on the bottom of shingle at 21 m depth. The pingers were spaced by 100 m, and the POD was placed next to the central pinger at 56°39.79′N, 5°53.67′W (Fig. 1). All pingers were attached 4 m off the bottom to simulate the height of a float line, and all PODs 2 m off the bottom. All devices were hauled every second week to replace batteries and download data.

An internal clock, developed for this study, turned the pingers on and off according to two preset schedules (the short cycle and the long cycle, respectively; Fig. 2). In Bloody Bay, three pingers at each end of the net were activated according to the short pinger cycle, while the central two pingers were always silent. The original objective of the silent pingers was to investigate the failure tolerance of pingers as a bycatch mitigation measure, i.e., if there would be a higher rate of animals crossing the virtual net line where the pingers were silent. However, as pingers were found to deter porpoises at greater distances than the size of the gap between the active pingers, this objective was not considered in the analyses. In Lagabay, pingers programmed with the two different cycles were alternated. Pingers with the long cycle were deployed between 20 June and 4 July and between 28 July and 7 August, while the pingers with the short cycle were deployed between 4 and 27 July. At both locations, the pingers were always started 2 h before high tide so that data were always collected during the same tidal phase.

In Bloody Bay, a four-person observation team surveyed the area: two scanners, a tracker, and a data recorder. The site was 80 m above sea level, and the position was 56°38.60′N, 6°06.70′W (Fig. 1). The observers did not know whether the pingers were active or silent. The two scanners searched the area for porpoise groups. One of them used naked eye, focussing on the area within 700 m, and the other used binoculars (7 × 50), focussing on the area between 700 and 1900 m from the observation site (Fig. 3). Vertical angles and horizontal bearings to harbour porpoise groups were determined using declinometers and horizontal angle boards. The tracker used a digital theodolite (Topcon total station GTS 212) to record the movements of the porpoise group that was closest to the simulated net. The

Fig. 1. Map showing the experimental setups and the locations of the study areas: Bloody Bay and Lagabay. The straight lines indicate the simulated nets; solid stars represent pingers that either emitted sound or were silent; open stars are constantly deactivated pingers; circles are porpoise detectors (PODs); and the diamond indicates the position of the observer team in Bloody Bay.



data recorder assisted the tracker and noted environmental data on, *e.g.*, visibility and sea state. The observer rotation and pinger schedules gave a maximum of 3 h of visual data per day. Only data collected during good visibility and sea state 0–3 were used in the analyses. Because of the high observation point, the tidal variation of the water level resulted in a small error in calculated surface positions. At the outer end of the simulated net, 5% of the tides caused an error greater than ±1 m, and the maximum error at spring flood was 6 m.

An overview of the aims, analyses, and data sources are given in Table 1. Based on the results of previous studies of free-ranging porpoises (Koschinski and Culik 1997; Cox et al. 2001; Culik et al. 2001), all statistical tests except those of group size and habituation were one-sided.

Echolocation encounter rate

Data from Bloody Bay, collected during the 4 h periods when pingers of the short cycle were either active or silent, were used for the analyses of echolocation encounter rate. The click train algorithm was found to occasionally split up long click trains into shorter segments, wherefore the rate of echolocation encounters was chosen as a measurement of echolocation activity. Echolocation encounters were defined as periods with trains that were separated by periods of silence with a minimum duration of 10 min. The echolocation

Fig. 2. Schedules of the short and long pinger cycles. Solid fields mean that pingers were active; open fields show that pingers were silent. The letters show when data were collected for the different analyses: (*a*) echolocation encounter rate, (*b*) initial sighting rate, (*c*) group size, (*d*) distance between porpoise track and simulated net, (*e*) return time, and (*f*) habituation. Impact refers to data collection periods after the pingers had been active and control to data collection periods after the pingers had been silent. The pingers were always silent during the impact and control periods.

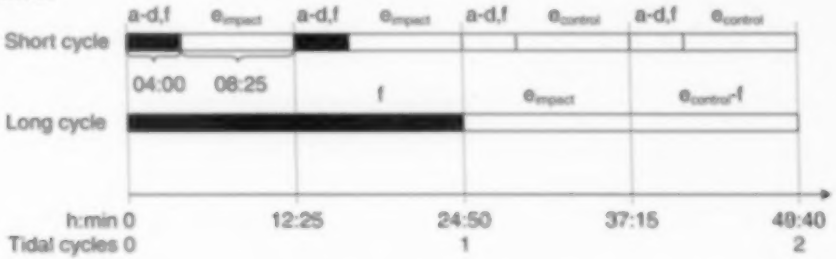
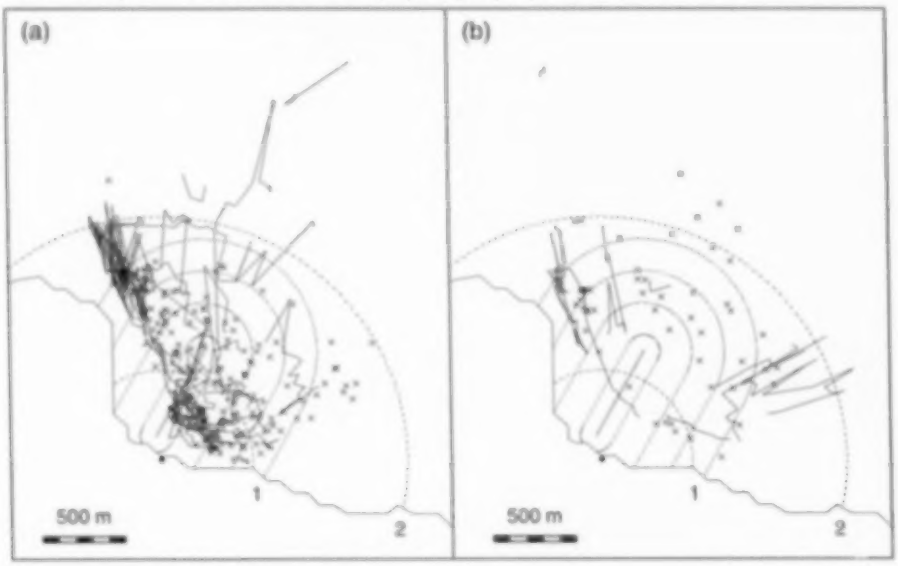


Fig. 3. Maps showing the visual setup and obtained visual data in Bloody Bay: (*a*) when the pingers were silent and (*b*) when the pingers were active. The position of the observer team is indicated by the solid diamond and the simulated net by the double line. The observer using naked eye to search for porpoises focussed her effort within the broken semicircle closest to the observation point (1), and the observer using binoculars focussed her effort in between the two broken semicircles (1 and 2). The distance zones used for the analyses of initial sighting rate and group size are indicated by solid, concentric lines around the simulated net. The positions of the initial sightings of harbour porpoise groups are indicated by × and the tracks of harbour porpoise groups by solid lines.



encounter rate was calculated as the number of encounters per hour.

Initial sighting rate and group size

Only data from Bloody Bay, collected during the 4 h periods when pingers of the short cycle were either active or silent, were used for the analyses of initial sighting rate and group size. The visual data was collected at the same time as the data for analyses of echolocation encounter rate, but the maximum duration of the sample unit was 3 h because of the observer rotation schedule. The duration of a sample unit was sometimes decreased by unfavourable weather conditions, and the minimum duration for a sample unit used in the analysis was 1 h. By dividing the number of initial sightings by hours of visual effort, it was corrected for different durations of the sample units. To identify the first sighting of each porpoise group, all sightings were pooled, likely duplicates and resightings of the same porpoise group were removed, and only the initial sightings were kept. Thereafter the positions of the initial sightings were placed into one of the following zones around the simulated net: 0–125, 125–375, 375–625, 625–875, and >875 m (Fig. 3). The sizes of the distance zones were chosen so that the PODs placed on the simulated net and at 250, 500, and 750 m from the net were encompassed by different zones.

Distance between porpoise track and simulated net

Only data from Bloody Bay, collected during the 4 h periods when pingers of the short cycle were either active or silent, were used for the analyses of distance between

Table 1. Overview of the aims, analyses, and data sources in the study.

Aim: Investigate the effect of pinger sound on ...	Analysis	Data source*
(*a*) echolocation encounter rate	Reduction at each POD	BB all PODs
(*b*) initial sighting rate	Reduction within each distance zone	BB scanners
(*c*) group size	Change within each distance zone	BB scanners
(*d*) distance between porpoise track and simulated net	Increase in minimum and average distance	BB tracker
(*e*) return time after pingers had been active or silent	Increase in time to first echolocation	BB PODs S Net and N Net, LB POD
(*f*) habituation	Correlation between effectiveness of pingers and start time of sample period	BB all PODs, LB POD

*PODs, porpoise detectors; BB, Bloody Bay; LB, Lagabay; S Net, POD at south end of simulated net; N Net, POD at north end of simulated net.

porpoise track and simulated net. From the recorded porpoise tracks, both minimum and average distances between the animals and the simulated net were calculated. Each track was considered to be an independent sample.

Return time

In the analyses of return time of porpoises, only data collected by PODs placed next to active pingers were used. For Bloody Bay, this implies only data from POD S Net and POD N Net (PODs at south end and north end of simulated net, respectively) were used, and these contained data collected during the operation of the short pinger cycle. The POD in Lagabay was also placed next to an active pinger, and this POD contained data collected during the operation of both the short and the long pinger cycle. In contrast with all other analyses, data for the analyses of return time of porpoises were always collected when the pingers were silent, after they had been either active (data collections period called impact) or silent (data collections period called control). If no echolocation occurred before the next active or silent period started, the total duration of the entire impact or control period was used instead. The total duration of both the impact and control periods was 12 h 25 min in the long pinger cycle and 8 h 25 min in the short pinger cycle (Fig. 2).

Habituation

Data from all PODs in both Bloody Bay and Lagabay were used for the analyses of habituation throughout the course of the experiment. The PODs in Bloody Bay contained only data collected during the short pinger cycle, while the POD in Lagabay contained data collected during both the short and the long pinger cycles. As the density of porpoises may have varied over the season because of non-experimental reasons, the effectiveness of pingers was calculated as the quotient between the echolocation encounter rate when the pingers were active and the echolocation encounter rate when then pingers were silent. A quotient was considered to be a sample unit, and it was analysed if the values of the quotients were correlated to the start times of the sample periods. A positive correlation would indicate a diminishing effectiveness of pingers over the season. For the short pinger cycle, each quotient was calculated as the total number of echolocation encounters in three consecutive active sample periods divided by the total number of echolocation encounters in the three consecutive sample periods that were closest in time. The summation over three sample periods was done to avoid the systematic error of excluding many data points with zero in the denominator. As the duration of one sample period in the short pinger cycle was 4 h, one quotient from the short pinger cycle contained data from 12 h when the pingers were active and from 12 h when the pingers were silent. For the long pinger cycle, only the second half of sample periods were used, as the echolocation rate in the beginning of the silent periods were likely to be affected by the pinger sound in the preceding active periods. As the duration of one sample period in the long pinger cycle was 24 h 50 min, one quotient from the long pinger cycle contained data from 12 h 25 min when the pingers were active and from 12 h 25 min when the pingers were silent. By using data from the same part of the long pinger cycle for both the active and silent periods, the duration of the sample period was equal, and the data were always collected during the same tidal phase (Fig. 2).

Results

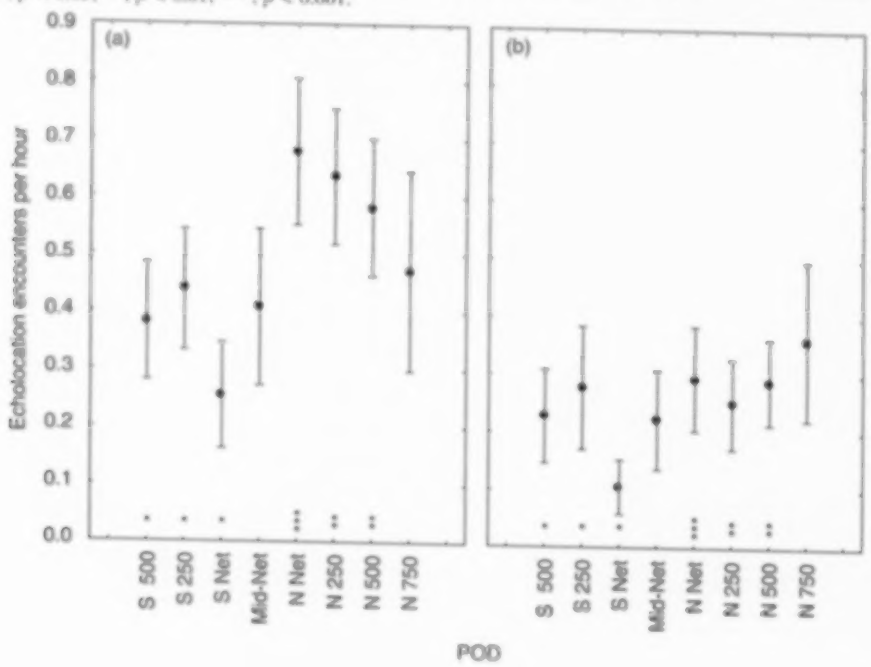
Echolocation encounter rate

In Bloody Bay, a total of 656 echolocation encounters were registered during a total of 1472 h of data logging (368 sample periods of 4 h each) at all PODs when the pingers were silent. When the pingers were active, a total of 334 echolocation encounters were registered during a total of 1352 h (338 sample periods of 4 h each). The median echolocation encounter rate per POD was 0–0.75 encounters per hour when the pingers were silent (maximum 1.50–2.25). In general, the echolocation encounter rate at the offshore PODs (POD N Net – POD N 750) was approximately twice as high as at the inshore PODs (POD Mid-Net – POD S 500). When pingers were active, the echolocation encounter rate was significantly lower at all PODs in Bloody Bay, except POD Mid-Net and POD N 750 (Table 2 and Fig. 4; one-sided Mann–Whitney *U* test adjusted for ties). This means that the sound of pingers reduced the echolocation encounter rate within the range of PODs placed perpendicular to the simulated net at distances up 500 m. POD Mid-Net was placed in between the two central pingers that were always silent and 150 m from the closest functional pinger on each side. For this POD, the p value was 0.090, which can be considered as a trend. For POD N 750, the p value was 0.250. Because of shorter battery life than expected, about as half as many samples were obtained from this POD as from the others.

Table 2. Comparisons of the number of echolocation encounters per hour between pingers active and pingers silent in Bloody Bay (one-sided Mann–Whitney *U* test adjusted for ties).

	Active		Silent			
POD	*n*	Median echolocation encounters·h^{-1} (quartile range)	*n*	Median echolocation encounters·h^{-1} (quartile range)	*Z*	*p*
S 500	45	0 (0–0.50)	49	0.25 (0–0.50)	2.02	0.021
S 250	45	0 (0–0.50)	49	0.25 (0–0.75)	2.28	0.011
S Net	45	0 (0–0.25)	49	0 (0–0.50)	1.92	0.027
Mid-Net	45	0 (0–0.25)	49	0 (0–0.75)	1.34	0.090
N Net	45	0.25 (0–0.50)	49	0.75 (0.25–1.00)	3.76	<0.001
N 250	43	0.25 (0–0.50)	48	0.50 (0.25–1.00)	4.06	<0.001
N 500	45	0.25 (0–0.50)	49	0.50 (0.25–1.00)	2.90	0.002
N 750	25	0.25 (0–0.75)	26	0.25 (0–1.00)	0.67	0.250

Fig. 4. Average echolocation encounter rate at each POD in Bloody Bay (*a*) when the pingers were silent and (*b*) when the pingers were active. The range bars indicate the two-sided 90% confidence interval. The asterisks indicate nonparametric differences between pingers active and silent: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.



Initial sighting rate

In Bloody Bay, a total of 158 porpoise groups were sighted during 49 h when the pingers were silent and 54 during 30 h when the pingers were active (Fig. 3). The median sighting rate for the entire scanned area (data pooled for all distance zones) was 1.25 groups per hour when the pingers were silent (range 0–7). In the distance zone 125–375 m from the simulated net, the sighting rate was significantly reduced by the sound of pingers. When the pingers were silent, the median sighting rate was 0.50 porpoise groups per hour (range 0–3.33). When the pingers were active, the median rate decreased to 0 (range 0–0.67) (one-sided Mann–Whitney *U* test adjusted for ties; $Z = -1.88$, $n_{active} = 10$, $n_{silent} = 18$, $p = 0.030$). This distance zone encompassed POD S 250 and POD N 250, i.e., the two PODs placed 250 m from the simulated net. In all other zones, the median sighting rate was 0 when the pingers were silent (maximum 1.33, 2.67, 1.33, and 3.59 porpoise groups per hour in zones 0–125, 375–625, 625–875, and >875 m, respectively), and no significant reduction was found when the pingers were active (one-sided Mann–Whitney *U* tests adjusted for ties; $Z \geq -1.17$, $n_{active} = 10$, $n_{silent} = 18$, $p > 0.05$).

Group size

The overall group size in Bloody Bay was 1.6 animals (standard deviation, SD = 0.82), and it did not change in any distance zone during pinger activity (analysis of var-

tance, ANOVA; $F_{[4,150]\ \text{interaction}} = 0.43$, $F_{[1,150]\ \text{pingers}} = 1.96$, $F_{[4,150]\ \text{distance zones}} = 2.60$, $p_{\text{interaction}}$, p_{pingers}, and $p_{\text{distance zones}} \geq 0.05$).

Distance between porpoise track and simulated net

In Bloody Bay, 35 harbour porpoise groups were tracked during 49 h when the pingers were silent and 11 during 30 h when the pingers were active (Fig. 3). Both minimum and average distances to the simulated net were significantly increased during pinger activity (one-sided Student's t test; $t_{\text{min}} = 1.93$, $t_{\text{average}} = 1.98$, $p_{\text{min}} = 0.03$, $p_{\text{average}} = 0.03$). On average, the minimum distance increased from 431 m (SD = 441) when the pingers were silent to 752 m (SD = 599) when the pingers were active. Similarly, the average distance increased from 653 m (SD = 432) to 961 m (SD = 516). When pingers were silent, a total of six tracks crossed the simulated net. On average, each of these tracks crossed the net 3.3 times (range 1–6). When the pingers were active, only one track crossed the simulated net once, and the point for crossing was in between the constantly silent pingers in the centre of the net. No statistical test was performed on these few data points.

Return time

During the operation of the long pinger cycle used in Lagabay, the time to the first echolocation encounter increased from 2 h 28 min (SD 3 h 35 min) in control periods (after pingers had been silent) to 6 h 02 min (SD 5 h 10 min) in impact periods (after pingers had been active). During the operation of the short pinger cycle, no significant delay was found at any location, neither in Lagabay nor at POD S Net or POD N Net in Bloody Bay. The statistics for the comparisons and the number of missing values, i.e., when no echolocation occurred during the control or impact period, are shown (Table 3). In Lagabay, the median echolocation encounter rate when the pingers were silent and when the presence of porpoises was not disturbed by previous pinger sound was 0.56 encounters per hour (maximum 1.67).

Habituation

There was a significant positive correlation of moderate strength between the effectiveness of pingers and the start time of the sample period at two inshore PODs in Bloody Bay: POD S 500 and POD S 250 (Fig. 5, Spearman rank order correlation; POD S 500: slope = 0.014, $R = 0.59$, $n = 14$, $p = 0.03$; POD S 250: slope = 0.023, $R = 0.63$, $n = 14$, $p = 0.01$). Further, there was a nonsignificant trend at two PODs on the simulated net: POD Mid-Net and POD N Net (POD Mid-Net: slope = 0.020, $R = 0.58$, $n = 11$, $p = 0.06$; POD N Net: slope = 0.001, $R = 0.51$, $n = 15$, $p = 0.05$). The average reduction in effectiveness of pingers from the start to the end of the experiment at these four PODs was 85%. At the remaining three PODs in Bloody Bay and the POD in Lagabay, no significant correlation or trend was found ($n_{\text{POD N 250}} = 14$, $n_{\text{POD N 500}} = 15$, $n_{\text{POD N 750}} = 8$, $n_{\text{LB}} = 14$, all $p > 0.05$).

Discussion

This is the first study using acoustic methods to investigate the spatial response of wild harbour porpoises on pinger sound. Furthermore, the impact of several pingers arranged on a bottom-set gill net was investigated, instead of looking at the impact of a single pinger placed close to the sea surface. The study demonstrates that the sound of Dukane pingers placed on a 700 m simulated gill net reduces the harbour porpoise echolocation encounter rate by 50%–100% at PODs placed up to 500 m away. A previous study has found a decrease by 84%, measured at the position of one Dukane pinger (Cox et al. 2001). The distance of 500 m corresponds to Trippel et al.'s (1999) presumption that Dukane pingers are clearly audible to porpoises at ranges up to 600 m in conditions with no rain and sea state 0–2. Nevertheless, the audible distance of pingers to porpoises in the present study is not known. This distance may be highly variable between areas and depends on many factors, such as number of pingers, pinger depth, bottom type, bottom topography, water depth, and water temperature.

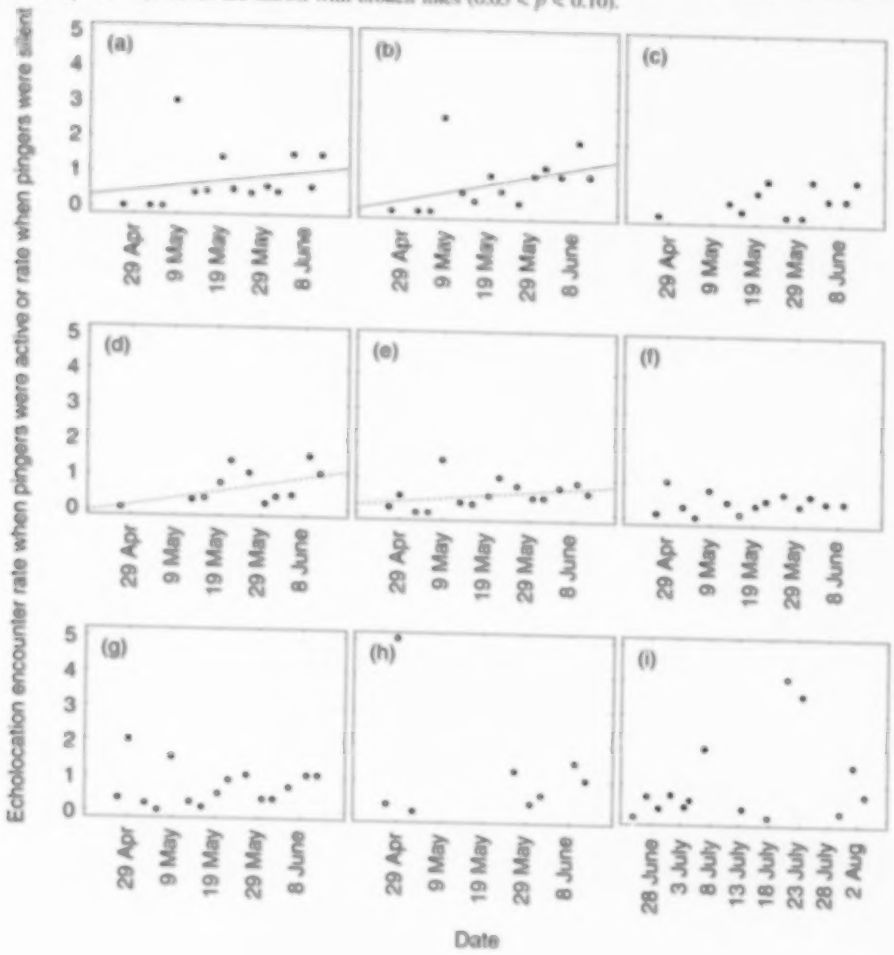
The harbour porpoise echolocation encounter rate was reduced at PODs placed up to 500 m from the simulated net, while the initial sighting rate of harbour porpoise groups were only reduced 125–375 m from the simulated net. The major explanation to the dissimilarity is probably that the acoustic setup was more efficient than the visual setup; therefore, it was easier to detect existing differences in the acoustic than in the visual data. As acoustic data were collected both at night and during the day, and visual data were only collected during the day, on average more than three times as many acoustic than visual samples were obtained. Further, the median echolocation encounter rate per POD was higher than the median rate of initial sightings in each distance zone. While the PODs continuously collected data from all present porpoise groups within their entire detection range, the observers could only search within a limited section of their zone at any time. The effect of pinger sound on echolocation encounter rate was not caused by a change in group size, as this was not affected by the pinger sound, but the effect may have been amplified by a reduction in individual echolocation rates of present porpoises. It has been suggested that wild harbour porpoises echolocate less frequently in the vicinity of an active pinger (Cox et al. 2001). In captivity, two harbour porpoises have been found to decrease their echolocation activity when exposed to three different pinger sound types. The animals responded most strongly to the initial presentations of a sound, and the reaction diminished rapidly in the following sessions (Teilmann et al. 2006). Yet another possibility is that the volume of water in which the PODs could detect porpoises may not

Table 3. Comparisons of the time to the first echolocation encounter after pingers had been active (impact) in relation to after they had been silent (control; one-sided Student's t test).

Bay POD	Pinger cycle	df	t	p	Replaced values impact	Replaced values control
LB	Long	19	1.82	0.04	3	1
LB	Short	42	0.28	0.39	1	3
BB S Net	Short	87	0.03	0.97	10	17
BB N Net	Short	87	1.08	0.28	18	3

Note: Replaced values are the number of impact or control periods that did not contain any echolocation encounter, and the total duration of the impact or control period was used instead of the time to the first encounter. LB, Lagabay; BB, Bloody Bay.

Fig. 5. Effectiveness of pingers (echolocation encounter rate when the pingers were active divided by the rate when the pingers were silent) throughout the course of the experiment at all PODs: (*a*) POD S 500, (*b*) POD S 250, (*c*) POD S Net, (*d*) POD Mid-Net, (*e*) POD N Net, (*f*) POD N 250, (*g*) POD N 500, and (*h*) POD N 750, all placed in Bloody Bay, and (*i*) the POD in Lagabay. The significant correlations are indicated by solid lines ($p < 0.05$); trends are shown with broken lines ($0.05 < p < 0.10$).



have been representative of the entire surface area of the visually scanned zones that they were placed in. For example, the surface area of the distance zone 375–625 m from the simulated net was more than five times greater than the sum of the 95% detection areas of the two PODs (areas calculated at the depth of the PODs) that were placed in this zone.

The sound of pingers increased the average distance of closest approach from 431 to 725 m. These distances are greater than reported in previous studies, but the displacement distance of approximately 300 m is of the same magnitude. Previous studies have observed an increase in average closest approach to one Dukane pinger from 220 to 428 m (Cox et al. 2001), an increase in closest surfacing position to one PICE pinger from 150 to 530 m (Culik et al. 2001), and an increase in average closest approach to one Lien pinger from approximately 30 to 133 m (Koschinski and Culik 1997). The longer distances of closest approach in the present study may have been caused by all the factors affecting the audible distance of pingers mentioned above. The distances may also have been affected by the density of porpoises in absence of pinger sound. If the density of porpoises in the strait just outside the bay normally was lower than the density of porpoises in the bay, then the average distance to the closest animal would have increased more than the deterrence distance of pingers on porpoises if the sound of pingers caused porpoises to leave the high density area. Further, the distances in closest approach may also have been influenced by the placement of the simulated net in a bay, as it primarily was accessible from 180° and not 360°. The difference in closest approach between Cox et al. (2001) and the present study, which both used the Dukane pinger, confirms Dawson's (1994) and Gearin et al.'s (2000) remark that it is important to replicate pinger studies

in both time and area. As in Cox et al. (2001), the high elevation of the observation point facilitated detection and accurate tracking of porpoise groups far away.

There was an element of habituation in Bloody Bay during the study period, although the effect was not uniform. The locations of the PODs with significant results or non-significant trends indicate that long-term habituation was more likely to happen (*i*) close to shore, where porpoise density was lower and animals may have passed through the sound of several pingers on their way in, or (*ii*) close to the pingers on the simulated net. Although the habituation effect did not occur as soon as in Cox et al. (2001) (50% reduction within 4 days of constant pinger operation), the results suggest that also intermittent exposure to pinger sound may cause habituation if the exposure is repeated over a long period of time. If Cox et al.'s results are put into context with the findings in return time, a long duration of pinger operation appears to produce both increasing habituation and increasing delay in return to a previously ensonified area.

The two central pingers on the simulated net were always silent, and POD Mid-Net was placed between these. The original objective of the constantly deactivated pingers was to test if this would lead to an increased bycatch risk at this position and thereby partly foil the decreased risk of bycatch in the rest of the net. However, this was never tested for, as pingers were found to reduce the echolocation encounter rate at substantially longer distances. At POD Mid-Net, there was a trend but no significant reduction in the echolocation encounter rate when the pingers were active. The only track that crossed the simulated net while the pingers were active did so once between the constantly silent pingers, but the number of data points on crossing tracks were too few to draw any firm conclusions. Therefore, our results neither confirm nor reject the hypothesis that pingers as a bycatch mitigation measure are sensitive to a few malfunctioning devices. These finding can be compared with the results of a study on the effect of spacing of Aquatec AQUAmark 100 pingers on the bycatch rate of harbour porpoises in the Danish North Sea fishery with bottom-set gill nets for European hake (*Merluccius merluccius*). According to the current EU legislation, the maximum spacing between pingers of the Dukane NetMark 1000 type is 100 m and between pingers of the Aquatec AQUAmark 100 type is 200 m (European Commission 2004). Compared with nets without pingers, the bycatch rate in the Danish experiment was reduced by 100% when pingers were spaced by 455 m and by 78% when pingers were spaced by 585 m (Larsen and Krog 2007). If the safety margins for malfunctioning pingers are not needed, and habituation is presumed to not increase the risk of bycatch, there are two alternative ways for the future development of pingers. Either the pinger effectiveness is kept constant, while the spacing is increased; this would be advantageous both economically and practically for the fisheries. Alternatively, the spacing is kept constant, while the effectiveness of pingers is reduced; this would reduce both the risk of harbour porpoises habituating to the sound of pingers and the risk of deterring porpoises from larger areas than needed to avoid bycatch.

Widespread use of pingers may not be suitable in coastal areas, as this may restrict the movements and distribution of harbour porpoises. An example can be given for the harbour porpoise in the Baltic region. The population structure in the region is not fully understood (Koschinski 2002; Berggren and Wang 2008; Palmé et al. 2008), but the harbour porpoise in the Baltic Sea is classified as critically endangered (CR) (Gärdenfors 2005). According to current EU legislation, pingers are mandatory for vessels of 12 m or over fishing with specified bottom-set gill nets in the western part of the southern Baltic Sea (ICES subdivision 24) and in the Kattegat and Skagerrak seas (ICES division IIIa), among other areas (European Commission 2004). The Baltic Sea connects to the Kattegat Sea through the Great Belt and the Sound. Following the ICES definitions that are used for the pinger legislation, the southern and northern openings of the Belt Sea are approximately 30 km wide, and the southern and northern openings of the Sound approximately 20 km wide. The Belt Sea includes the Little Belt, the Great Belt, the Kiel Bight, and the Mecklenburger Bight. To pass from the Baltic Sea to the Kattegat Sea, a harbour porpoise has to swim through the Little Belt, which is 700 m wide, the Great Belt, which is 10 km wide, or the Sound, which is 3.5 km wide, at the narrowest part. The widths of the borders between the seas and the straits within the seas are comparable with those in the present study; the sound of pingers significantly reduced the echolocation encounter frequency of harbour porpoises within the radial distance of 500 m. Based on historical catch statistics from Danish waters, it has been presumed that there was an extensive migration of harbour porpoises out of the Baltic Sea during the winter until the end of the 19th century (Andersen 1982; Kinze 1995). Such a migration pattern would decrease the risk of ice entrapment in the Baltic Sea under severe ice conditions. Larger numbers of dead porpoises have been reported from the island of Bornholm in the southern Baltic Sea after severe ice conditions in February 1929 and in the waters west of the island of Als in the Little Belt after severe ice conditions in January 1942 (Lockyer and Kinze 2003). More recent data on the movements of harbour porpoises have been obtained from 31 harbour porpoises equipped with satellite transmitters in the Danish parts of Belt Sea and the southern Kattegat Sea. More than two-thirds of these animals moved among the Belt Sea, the Kattegat Sea, and (or) the southern Baltic Sea, although only one animal swam a longer distance into the southern Baltic Sea (Teilmann et al. 2004). Based on data on movements and abundance, 16 high density areas of porpoises have been identified in Danish waters, and their importance has been ranked on a three-graded scale (Teilmann et al. 2008). Seven of the identified areas are entirely or partly within the waters where pingers are mandatory according to the EU regulation (European Commission 2004), and seven are entirely within the Belt Sea. If pingers were widely used at the borders between these waters, or in the narrow straits of the Belt Sea or the Sound, the movements and distribution of harbour porpoises might be adversely affected, and the already serious situation for the harbour porpoise in the Baltic Sea may be aggravated.

In conclusion, the present study gives an example of how a deployment of a typical bottom-set gill net equipped with Dukane pingers affects the distribution of harbour porpoises in time and space. It reveals that the sound of pingers re-

duces porpoise abundance at a greater spatial range than previously known, that the return time of porpoises to previously ensonified areas increases with longer exposure to pinger sound, and that porpoises can habituate to pinger sound even if pingers are used only intermittently. This implies that pingers have a greater potential for exclusion of porpoises from fishing areas than previously recognised, although this exclusion zone is likely to diminish if habituation develops. Further, the results confirm that pingers are an effective bycatch mitigation measure, but it is not clear how sensitive the measure is to a few malfunctioning devices. While pingers may be appropriate to reduce the number of harbour porpoise bycatches in sparsely distributed offshore fisheries that are dynamic in time and space, alternative long-term solutions should be sought for migration routes and critical habitats.

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Genetic variability in reaction norms between farmed and wild backcrosses of Atlantic salmon (*Salmo salar*)

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Abstract: Adaptive responses to environmental heterogeneity may vary among populations. Genetic variability in reaction norms might account for population differences in the ability to respond to environmental change and may reflect local adaptation. Reaction norms for early life history traits were compared among three population crosses of Atlantic salmon (*Salmo salar*). Two comprised second-generation backcrosses introgressed with either farmed or wild genes; the third comprised individuals from a second-generation, pure wild cross. Using a common-garden experimental protocol, each cross was exposed to three temperature regimes. Plasticity in embryonic development, growth, survival, and body size was measured from fertilization up to 24 weeks of exogenous feeding. Reaction norms differed markedly among crosses, irrespective of whether individuals interbred with those whose genes originated from another wild population or from a cultured population. We find that introgression involving individuals with comparatively few genetic differences can change reaction norms. If plasticity represents an adaptive response to local environments, then changes to reaction norms resulting from interbreeding between populations are unlikely to have a beneficial effect on fitness.

Résumé : Les réactions adaptatives à l'hétérogénéité de l'environnement peuvent varier d'une population à une autre. La variabilité génétique des normes de réaction peut expliquer les différences entre les populations dans leur capacité à réagir aux changements environnementaux et peut représenter une adaptation locale. Nous avons comparé les normes de réaction des traits du début du cycle biologique chez trois populations croisées de saumons atlantiques (*Salmo salar*). Deux des populations comprenaient des individus rétrocroisés de seconde génération avec introgression de gènes de pisciculture ou de gènes sauvages; la troisième comprenait des individus de seconde génération issus d'un croisement sauvage pur. Dans un protocole expérimental de jardin commun, chaque croisement a été exposé à trois régimes thermiques. Nous avons mesuré la plasticité du développement embryonnaire, de la croissance, de la survie et de la taille corporelle depuis la fécondation jusqu'après 24 semaines d'alimentation exogène. Les normes de réaction varient de façon marquée d'un croisement à l'autre, indépendamment du fait que les individus aient été croisés avec d'autres dont les gènes provenaient d'une autre population sauvage ou d'une population d'élevage. Nous observons que l'introgression, qui implique des individus avec comparativement peu de différences génétiques, peut modifier les normes de réaction. Si la plasticité représente une réaction adaptative aux environnements locaux, alors les changements de normes de réaction dus aux croisements entre les populations sont peu susceptibles d'avoir un effet bénéfique sur la fitness.

[Traduit par la Rédaction]

Introduction

Phenotypic plasticity reflects the ability of individuals to respond to environmental change (Bradshaw 1965), a characteristic of presumed fundamental importance to the persistence of natural populations (Schlichting and Pigliucci 1998). Given that the direction of selection can vary in both space and time in heterogeneous environments (Via and Lande 1985), the ability of a genotype to produce different phenotypes can allow for short-term, potentially adaptive responses to environmental change (Pigliucci 2001).

Plasticity can be heuristically and graphically represented by a norm of reaction, defined as the profile of phenotypes produced by a given genotype across an environmental gradient (Schmalhausen 1949). Reaction norms provide information on the magnitude of trait plasticity, the presence of genotype × environment interactions on the phenotypic expression of a given trait, and the extent to which the additive genetic variance of a trait changes with the environment (de Jong 1990; Falconer and Mackay 1996). Population-level differences in reaction norms may explain why populations respond differently to natural or anthropogenic changes in the environment (Schlichting and Pigliucci 1998; Dieckmann and Heino 2007) and might reflect local adaptation. If true, then changes to either the elevation or slope of reaction norms could have negative consequences for fitness.

Within this context, we explored the degree to which interbreeding alters the shapes of reaction norms in Atlantic salmon (*Salmo salar*), a species whose life history varies considerably throughout its geographical range (Hutchings

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and Jones 1998). Although differences in the average expression of various traits have been hypothesized to reflect local adaptation (Taylor 1991; McGinnity et al. 2003; Garcia de Leaniz et al. 2007), population differences in life-history reaction norms have not been previously examined in this species, with the exception of those recently documented for male parr maturity (Piché et al. 2008).

The potential for populations to differ in their ability to respond to environmental change may be particularly important from a conservation perspective. There is considerable evidence that deliberate or inadvertent introduction of cultured (e.g., farmed, hatchery) salmon to the wild can lead to evolutionary change in native populations (reviewed by Hutchings and Fraser 2008). As a consequence, cultured salmon may significantly alter the genetic composition of native populations and affect their ability to respond adaptively to their local environments (Hindar et al. 1991; Gharrett and Smoker 1993). For example, interbreeding between populations may result in the breakdown of coadapted gene complexes, leading to a reduction in fitness within hybrid lineages attributable to outbreeding depression (Granath et al. 2004; McClelland et al. 2005; Edmands 2007).

One means of exploring the degree to which interbreeding might affect plasticity, and thus fitness, would be to compare the shapes of life history reaction norms between a pure population cross and those hybridized with the pure cross, using a common-garden experimental protocol. We adopted this approach, using three population crosses of Atlantic salmon. The pure cross was one generation removed from the wild. The others were second-generation backcrossed populations possessing introgressed genes from either a cultured or a nonlocal wild population. Backcrossed salmon were used because this particular genetic makeup can be considered representative of what may be evident in the wild given the potential for escaped farmed or introduced nonlocal salmon to interbreed successfully with native populations (Lura and Sægrov 1991; Crozier 1993, 2000).

Few studies on fishes have distinguished genetic and environmental variation in the average expression of traits among second-generation hybrids (McGinnity et al. 2003; Smoker et al. 2004; Tymchuk et al. 2007) and only one — Fraser et al.'s (2008) study of acidity tolerance — has examined the effects of introgression on the shapes of reaction norms. Given its influence on development in early life in ectotherms in general (Einum and Fleming 2000; Ojanguren and Braña 2003) and in salmonid fish in particular (Alderdice and Velsen 1978; Beacham and Murray 1987; Hebert et al. 1998), we use variation in temperature to construct an environmental gradient against which reaction norms for life history traits can be compared.

The objectives of the present study are twofold: (*i*) to determine the effects of introgression on the expression of early life history traits across temperatures, and (*ii*) to examine whether introgression affects the shapes of reaction norms for early life history traits in Atlantic salmon.

Material and methods

Establishment of population crosses

The second-generation (F_2) experimental fish were derived from first-generation (F_1) progeny whose parents originated from three different populations. Two of these consisted of wild Atlantic salmon in Stewiacke River (S; 44°59′N, 64°05′W) and Tusket River (T; 43°53′N, 65°56′W), Nova Scotia, Canada. The salmon inhabiting Stewiacke River are among those comprising the Inner Bay of Fundy unit (more than 30 rivers in total; Fisheries and Oceans Canada (DFO) 2003), which has been assessed as endangered by Canada's national science advisory body on species at risk (Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006). Water temperatures experienced by salmon in the Inner Bay of Fundy rivers are considerably cooler than those experienced by salmon in Tusket River. Based on data recorded during the summer (15 June – 5 September) in either 2000 or 2001, average water temperature in four Inner Bay rivers is 2.5 to 5.8 °C cooler than that in Tusket River (MacMillan et al. 2005).

The third population was of cultured, or farmed (F), origin and was obtained from the Atlantic Salmon Broodstock Development Programme (St. Andrews, New Brunswick (NB)). The progenitors of these salmon were from the Saint John River, NB (45°22′N, 66°01′W) (Glebe 1998). It has been estimated that these fish have been subjected to artificial and domestication selection for four generations (Glebe 1998). Empirical genetic data indicate that these populations are genetically distinct from one another (McConnell et al. 1997; Verspoor et al. 2002). The original crosses from which the F_1 progeny were produced were made in November 2001, at which time three pure (T × T, S × S, and F × F) and three hybrid crosses (T × S, T × F, S × F) were created in the Aquatron Facility at Dalhousie University (Lawlor et al. 2009). Crosses were then reared in a common environment for one generation, which served to eliminate the potential for residual environmental effects to generate population differences. Each of these six population crosses was the product of 10 different full-sib families, as described by Lawlor et al. (2009).

The F_2 crosses used in the present study were produced in the Aquatron Facility on 1 and 9 December 2004. The three populations consisted of one pure cross and two backcrosses. The pure population was an F_2 laboratory population that originated from the Tusket River (TT × TT). The first backcross comprised Tusket River salmon and F_1 hybrids from Tusket and Stewiacke rivers (TT × ST). The second backcross comprised Tusket River salmon and F_1 hybrids of Tusket River and farmed salmon (TT × FT). Eight males and eight females (all of the same age) from the F_1 populations were randomly selected to create each population cross (for details, see Table 1). Upon fertilization, the eight individual families were combined by population cross in incubator trays, and unfertilized eggs were discarded. They were held in the dark, undisturbed in flowing water, for 1 day. Thereafter, 4200 eggs counted from each cross were randomly selected, using a cup to collect multiple eggs at once, and moved to their assigned tanks. At the time of fertilization, individual parental data (length and weight) were recorded, and 50 fertilized eggs were randomly sampled from each population to determine egg size at fertilization.

Reaction norms were constructed during two separate common-garden experiments. The first was undertaken dur-

Table 1. Breeder framework for the second-generation crosses of Atlantic salmon: Tusket pure cross and Tusket × (Stewiacke × Tusket) performed 1 December 2004; Tusket × (farmed × Tusket) backcross performed 9 December 2004.

Tusket pure cross	Tusket × (Stewiacke × Tusket) backcross	Tusket × (farmed × Tusket) backcross
TT♀1 × TT♂1	ST♀1 × TT♂1	FT♀1 × TT♂1
TT♀2 × TT♂2	ST♀2 × TT♂2	FT♀2 × TT♂2
TT♀3 × TT♂3	ST♀3 × TT♂3	FT♀3 × TT♂3
TT♀4 × TT♂4	ST♀4 × TT♂4	FT♀4 × TT♂4
TT♀5 × TT♂5	TT♀1 × ST♂5	TT♀1 × FT♂5
TT♀6 × TT♂6	TT♀2 × ST♂6	TT♀2 × FT♂6
TT♀7 × TT♂7	TT♀3 × ST♂7	TT♀3 × FT♂7
TT♀8 × TT♂8	TT♀4 × ST♂8	TT♀4 × FT♂8

ing the embryonic stage, which extended from fertilization until absorption of the yolk sac. The second experiment focused on the early life feeding stages of development, i.e., during the first 24 weeks of exogenous feeding. To a first approximation, the temperatures to which the salmon were exposed during the embryonic and early life feeding experiments encompassed those that these fish might reasonably be expected to experience in the wild during these developmental stages.

Experimental protocol: embryonic stage

Eggs from each population cross were reared under identical conditions in twenty-four, 85 L circular tanks (0.48 m wide, 0.47 m high) at three different temperature regimes (mean ± standard deviation, SD): low (5.3 ± 0.1 °C), medium (9.2 ± 0.1 °C), and high (11.7 ± 0.1 °C). There were three replicate tanks, each containing 300 eggs, for each cross at the high- and medium-temperature treatments and two replicates for each cross at the low-temperature treatment. Eggs were held in the dark to mimic the light conditions that they would be expected to experience in the natural environment. Temperatures were recorded daily and dead eggs were removed periodically, with care taken to minimize disturbance.

To quantify egg size, 50 eggs from each population cross were randomly collected at the time of fertilization, evenly distributed into 10 containers, and dried at 40 °C for 4 days. We then compared the mean dried egg weight per container among crosses.

Survival during the embryonic stage was defined as the proportion of fertilized eggs that survived until the date on which the last eggs hatched (17 March 2005). Eggs were checked after fertilization approximately once weekly and dead eggs were removed periodically. Once the embryos had reached the eyed stage, dead eggs were removed daily to prevent the spread of fungus. Upon hatching, in addition to recording the date and time of hatch, individuals were euthanized with eugenol (Sigma-Aldrich, Oakville, Ontario), and a maximum of four alevins were placed in 1.5 mL microtubes and preserved in 65% ethanol for similar time periods prior to analysis. Preserved individuals were measured and weighed to determine body length at hatch and yolk sac weight at hatch. Fifty individuals were measured from each replicate tank. Length at hatch was measured with vernier calipers to the nearest 0.1 mm. Yolk sac weight at hatch was measured to the nearest 0.001 g. To measure yolk sac weight, the yolk sac was removed from the rest of the body and weighed separately.

Experimental protocol: early life stage

At fertilization, 1500 eggs from each population were randomly selected and divided evenly among five 100 L circular tanks (n = 15 tanks of 0.66 m diameter and 0.43 m height). Throughout the embryonic stage, eggs were held in the dark at approximately 5 °C. Once all the eggs had hatched, but prior to swim-up, individuals were transferred to twenty-four, 85 L circular tanks (diameter, 0.48 m; height, 0.47 m). Temperature in all 24 tanks was increased to 13.7 ± 0.4 °C to initiate swim-up. After 1 week, three different temperature regimes were established: low (15.4 ± 1.3 °C), medium (18.6 ± 0.7 °C), and high (21.8 ± 0.7 °C). There were three replicates at each of the high- and medium-temperature regimes, and two replicates at the low-temperature regime. Temperature in each tank was recorded daily. Individuals were divided evenly into the designated tanks, resulting in initial population sizes of 102 fish in each of the TT × TT tanks, 125 in each of the TT × ST tanks, and between 75 and 100 fish in each of the TT × FT tanks.

Throughout the early life stage, fish were exposed to a 12 h light – 12 h dark photoperiod cycle. The diet consisted of a commercial fish feed (Corey High Pro starter diet; Corey Aquafeeds, Fredericton, NB). Fish in each tank were initially fed six times daily during daylight hours, a frequency that was reduced gradually to three times daily by the 45th day after the initiation of exogenous feeding. The size of each food item gradually increased from 0.5 to 1.0 mm; the volume of food was based on ensuring that all fish were fed to satiation. Fish were considered satiated if there was evidence of uneaten food at the bottom of the tanks; uneaten food and other waste materials were removed once daily.

Survival during the early life stage was quantified from the onset of exogenous feeding until 11 weeks thereafter. Although the experiments continued until the end of the 24th week, a mass-mortality event during the 11th week in one of the TT × FT low temperature treatment tanks (a consequence of nonbiological factors) necessitated the quantification of survival from the onset of feeding until this date.

Fish were measured for length and weight approximately once monthly (six sampling periods in total). Sampling was performed by lightly anaesthetizing (with eugenol) 50 individuals from each of the 24 tanks and then obtaining an image of two to four individuals at a time, using a Sony Mavica digital camera located at a constant distance from the fish. To minimize handling time, 15 of the 50 fish were randomly subsampled and individually weighed to the nearest 0.1 g. Following recovery, fish were returned to their designated tanks. Following Ricker (1979) and Haugen and Vollestad (2000), specific growth rates were calculated for each individual as

$$(1) \qquad G = (\ln(L_2 - L_1)/(t_2 - t_1)) \times 100$$

where L_x is fork length (mm) at time t_x (estimated from the digital images to the nearest 0.1 mm) and t is measured in days.

Table 2. Mean (± standard deviation, SD) parental fork length, weight, and egg weight of female breeders used to produce the experimental, second-generation crosses of Atlantic salmon (T, Tusket River; S, Stewiacke River; F, farmed salmon).

	TT females 1–4	TT females 5–8	ST females 1–4	FT females 1–4
Fork length (cm)	57.25±2.87	54.75±0.50	53.00±3.56	55.25±2.36
Weight (g)	2867.00±437.28	2279.50±70.24	2530.00±671.08	2393.50±325.46
Egg dry weight (g)*	$3.8\times10^{-2}\pm0.2\times10^{-2}$		$3.6\times10^{-2}\pm0.8\times10^{-2}$	$4.4\times10^{-2}\pm0.2\times10^{-2}$

*Calculated as TT × TT, ST × TT, and FT × TT egg dry weight (g).

Fig. 1. Reaction norms for (*a*) proportion surviving to hatch, (*b*) days to 50% hatch, (*c*) length at hatch, (*d*) yolk sac weight, (*e*) survival from hatch until 11 weeks after the initiation of exogenous feeding, (*f*) fork length at week 24, (*g*) weight at week 24, (*h*) specific growth rate for three population crosses of Atlantic salmon (*Salmo salar*) reared at three temperature regimes: low (5.3 °C for *a* through *d*; 15.4 °C for *e* through *h*), medium (9.2 °C for *a* through *d*; 18.6 °C for *e* through *h*), and high (11.7 °C for *a* through *d*; 21.8 °C for *e* through *h*). Population abbreviations: T, Tusket River; S, Stewiacke River; F, farmed origin. The reactions norms, showing population means ±1 SE, corresponding to each population are indicated as follows: TT × TT, ■; TT × ST, ●; TT × FT, ▲.

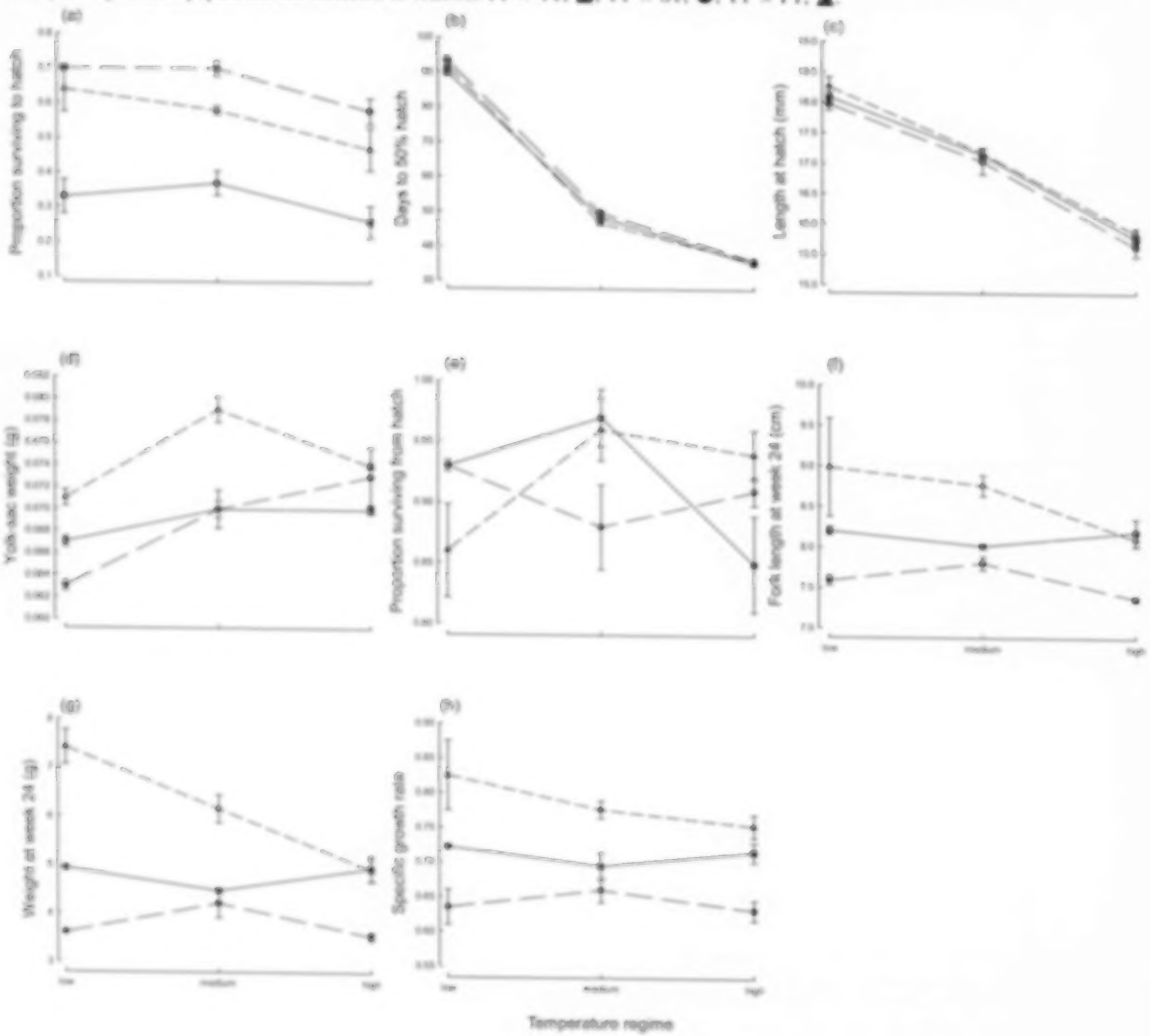


Table 3. Summary of two-way analysis of variance (ANOVA) statistics (F value with degrees of freedom in parentheses) for the effects of population, temperature, and population–temperature interactions (Pop × Temp) on several phenotypic traits.

	Effect		
Trait	Population	Temperature	Pop × Temp
Proportion hatched	60.54 (2, 19)*	9.39 (2, 19)*	0.35 (4, 15)
Days to 50% hatch	18.99 (2, 15)*	17233.57 (2, 15)*	9.94 (4, 15)*
Length at hatch	1.98 (2, 19)	259.24 (2, 21)*	0.11 (4, 15)
Yolk sac weight	19.58 (2, 15)*	12.63 (2, 15)*	4.04 (4, 15)*
Survival from hatch to week 11	0.66 (2, 15)	1.51 (2, 15)	2.71 (8, 15)*
Fork length at week 24	15.61 (2,21)*	3.45 (2, 15)	2.92 (6, 15)
Weight at week 24	10.39 (2, 21)*	1.49 (2, 15)	1.64 (6, 15)
Specific growth rate	32.55 (2, 21)*	1.13 (2, 15)	1.43 (6, 15)

*$P < 0.05$.

Statistical analysis

Among-population differences in trait means and population × temperature interactions (Pop × Temp) were examined using two-way factorial analyses of variance (ANOVAs; Zar 1999; Crawley 2002). Temperature (low, medium, and high), population (TT × TT, TT × ST, and TT × FT), and interactions among populations were set as fixed terms. Given that fish weight at week 24 was not normally distributed, we used a generalized linear model with a gamma distribution to analyse these data. Differences in egg size and parental female size were examined using a one-way ANOVA with a Tukey post-hoc test.

Results

Embryonic stage

The average size of egg from which the progeny were produced differed among population crosses. These differences, however, could not be attributed to differences in female body size, the primary correlate of egg size in fishes (Wootton 1998) (Table 2). Although eggs produced by TT × FT females were significantly heavier than those produced by females from the two other populations ($F_{[2,27]}$ = 21.70, $p < 0.0001$), neither maternal length nor weight differed among populations (one-way ANOVA for TT females 5–8, ST females 1–4, and FT females 1–4; length, $F_{[2,11]}$ = 0.91, $p = 0.44$; weight, $F_{[2,11]} = 0.34$, $p = 0.72$).

Survival to hatch differed significantly among populations (Fig. 1*a*; Table 3), being lowest at all temperatures among TT × TT progeny and highest among the TT × ST progeny (low temperature, $F_{[2,5]} = 16.86$, $p = 0.023$; medium temperature, $F_{[2,8]} = 46.95$, $p < 0.001$; high temperature, $F_{[2,8]}$ = 10.76, $p = 0.01$). Survival varied with temperature (Table 3), although the Pop × Temp term was not significant. Thus, although the elevation of the reaction norms differed among crosses, the slopes did not.

Elapsed time until 50% hatching was influenced by temperature, population, and an interaction between temperature and population (Fig. 1*b*; Table 3). Within each population, the elapsed time was inversely related to water temperature (TT × TT, $F_{[2,7]}$ = 2800, $p < 0.0001$; TT × ST, $F_{[2,7]}$ = 8851, $p < 0.0001$; TT × FT, $F_{[2,7]}$ = 15 080, $p < 0.0001$). Time to 50% hatch was also significantly influenced by a population–temperature interaction ($p = 0.003$), indicating that the slopes of the reaction norms differed among crosses.

At hatching, length did not differ among population crosses (Fig. 1*c*; Table 3). Yolk sac weight, however, was significantly influenced by an interaction between population and temperature ($p = 0.02$; Fig. 1*d*; Table 3), indicative of differences in reaction norm slopes for yolk sac weight among crosses. In general, TT × FT individuals possessed the largest yolk sacs.

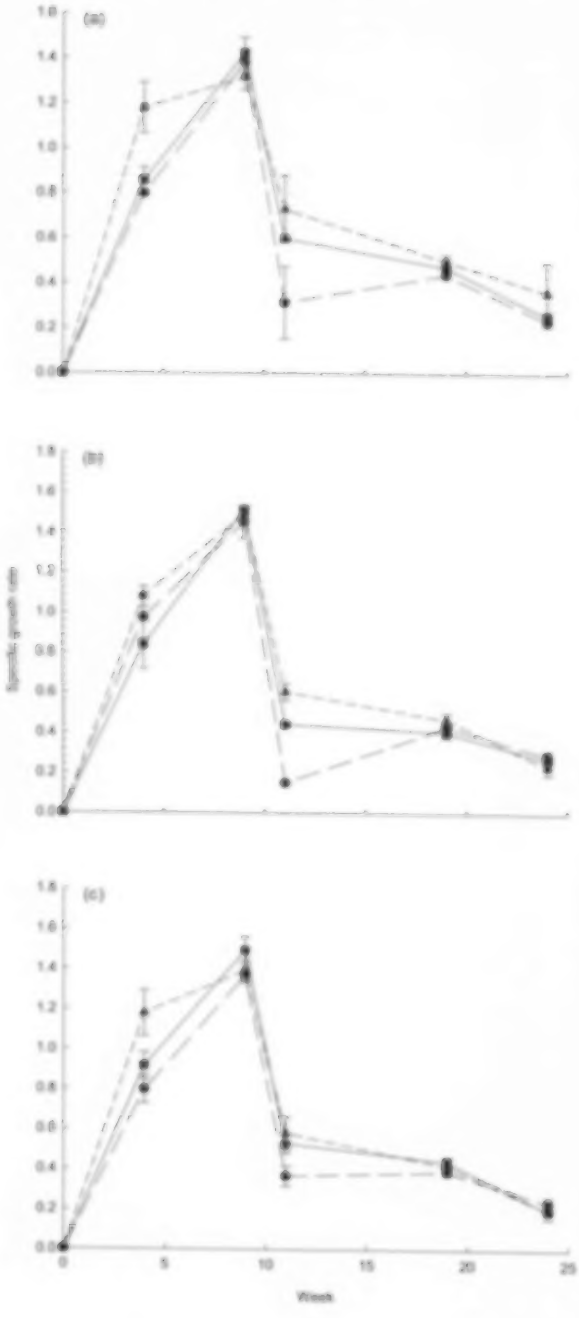
Early life stage

Reaction norms for posthatch survival differed among population crosses, as reflected by a significant interaction between population and temperature ($p = 0.015$; Fig. 1*e*; Table 3). These among-population final differences in survival could not be attributed to final differences in density (r = 0.024, $N = 23$, $p = 0.47$).

After yolk sac absorption, the average length of individuals from the TT × FT population was marginally, albeit significantly, less than that of the other two populations (TT × TT = 2.6 ± 6.7 × 10^{-3} cm; TT × ST = 2.6 ± 6.8 × 10^{-3} cm; TT × FT = 2.5 ± 6.2 × 10^{-3} cm; $F_{[2,1199]} = 17.41$, $p <$ 0.0001). At 24 weeks after hatching, there were significant differences in the elevation of reaction norms for body size. Progeny from the TT × FT population cross possessed the greatest length and weight at both the low- and medium-temperature regimes (Figs. 1*f*, 1*g*), but not at the high-temperature regime (body length: low temperature, $F_{[2,3]}$ = 4.32, $p = 0.13$; medium temperature, $F_{[2,6]} = 24.15$, p = 0.0014; high temperature, $F_{[2,6]} = 19.58$, $p = 0.002$; body weight: low temperature, $F_{[2,3]} = 1.84$, $p = 0.3$; medium temperature, $F_{[2,6]} = 19.74$, $p = 0.0023$; high temperature, $F_{[2,6]} = 15.03$, $p = 0.0046$; Table 3).

Measured on a monthly basis, specific growth rate differed among populations and was highest, at every temperature, for progeny from the TT × FT population (low temperature, $F_{[2,3]} = 10.25$, $p = 0.046$; medium temperature, $F_{[2,6]} = 12.4$, $p = 0.0074$; high temperature, $F_{[2,6]} = 16.93$, $p = 0.0034$; Fig. 2). Reaction norms for growth differed in elevation among population crosses but not in slope (Fig. 1*h*; Table 3). There was no influence of density on growth rate (i.e., length at age) based on the results of a three-way ANOVA treating density as a fixed effect ($F_{[1,8]}$ =

Fig. 2. Temporal variation in specific growth rate experienced by three Atlantic salmon (*Salmo salar*) population crosses (TT × TT, ■; TT × ST, ●; TT × FT, ▲; T, Tusket River; S, Stewiacke River; F, farmed origin) from the 1st to the 24th week of exogenous feeding: (*a*) low temperature regime, 15.4 °C; (*b*) medium temperature regime, 18.6 °C; (*c*) high temperature regime, 21.8 °C.



4.21, $p = 0.07$), although density did influence final body weight ($F_{[1,6]} = 118.37$, $p < 0.0001$).

Discussion

The present study provides evidence of genetic variability in fitness-related traits and their plastic responses to changes in temperature between second-generation backcrossed Atlantic salmon and one of the original parental populations. These differences in plasticity are consistent with the hypothesis that introgression can affect population responses to environmental change by altering the elevation and slopes of reaction norms for survival, growth, and metrics thereof in early life. By altering the shapes of reaction norms, interbreeding between first-generation hybrids and their parental population, a level of introgression involving comparatively small genetic differences between parents, has the potential to influence individual fitness in wild populations (Fraser et al. 2008).

Changes to reaction norms are evident irrespective of whether individuals interbreed with those whose genes originate from other wild populations or from populations that have been reared in a cultured environment. Nonetheless, there was some consistency in the disparity in reactions norms among crosses. For several traits (e.g., yolk sac weight, size at week 24, specific growth rate), the reaction norm for the pure TT × TT cross was intermediate relative to those of the two second-generation backcrosses. Among these same traits, reaction norms expressed by the wild hybrid population (TT × ST) tended to reflect lower trait values across temperature regimes relative to those expressed by the other two crosses. However, these patterns were not consistent among all traits, underscoring the unpredictability of the effects of hybridization on fitness-related traits.

Some of the differences in reaction norms resulting from introgression may be attributable to outbreeding depression, although a direct comparison between pure parental and second-generation backcrosses would be required to test this hypothesis (Lynch and Walsh 1998; McClelland et al. 2005; Tymchuk et al. 2007). All else being equal, outbreeding depression is expected to reduce fitness within hybrid populations, relative to the pure parental populations, because of the breakdown of co-adapted gene complexes. This might provide an explanation for the lower-elevation reaction norms — resulting in smaller sizes, longer hatching times, and slower specific growth rates — observed among TT × ST salmon. The absence of a similar response among TT × FT salmon might be attributable to strong directional selection resulting from domestication practices that typically select for increased growth and body size in culture environments (Einum and Fleming 1999; Hutchings and Fraser 2008).

Nongenetic influences on offspring size can translate into differences in offspring size and survival, resulting in an overestimation of genetic variance and the interpretation of environmentally based maternal effects as genetic effects (Falconer and Mackay 1996). Although we cannot entirely discount the possibility that our results might have been influenced by maternal effects, our experimental protocol was intended to minimize their influence. Firstly, the body size of parental females, a primary correlate of differences in

egg size (Wootton 1998), did not differ among population crosses. Secondly, half of the females involved in each cross were involved in all other crosses, reducing further the possibility that differences among populations could be attributable to individual female effects. Although the TT × FT eggs were significantly larger than those produced by the other parental females (despite the absence of differences in female body size), there were no significant differences among crosses in length at hatch, the developmental stage at which maternal effects are expected to be most pronounced (Heath et al. 1999; Einum and Fleming 2000). Furthermore, the average length of TT × FT individuals at first feeding was significantly less than those of the other two populations, indicating that the increased yolk sac size of the TT × FT population did not contribute to an increased size prior to the beginning of exogenous feeding.

The existence of genotype × environment interactions, reflected herein by interactions between population and temperature, implies that there can be genetic variation for plasticity among fish populations (Dieckmann and Heino 2007; Hutchings et al. 2007; Piché et al. 2008). The existence of such genetic variability might facilitate adaptation by populations to their local environments. Evidence that populations "perform" best (e.g., experience the fastest growth rate, highest survival) at the temperatures that they are most likely to experience in the wild would provide indirect evidence of such local adaptation (Haugen and Vøllestad 2000; Hutchings et al. 2007; Fraser et al. 2008).

In summary, we find that introgression can change the shapes of life history reaction norms in Atlantic salmon. Although these changes could potentially affect the ability of populations to respond adaptively to environmental change, our results underscore the difficulty in reliably predicting the demographic consequences of interbreeding (Weir and Grant 2005), irrespective of whether the introgression occurs with cultured individuals. Nonetheless, our observation that comparatively small genetic differences between breeding individuals can significantly affect reaction norms for traits closely related to fitness does raise questions from a conservation perspective. If their shapes in wild populations represent adaptive responses to local environments, then one might reasonably predict that changes to reaction norms are unlikely to have a positive influence on fitness (Fraser et al. 2008).

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A comparison of the heat shock response in juvenile and adult rainbow trout (*Oncorhynchus mykiss*) — implications for increased thermal sensitivity with age

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Abstract: We conclude that the heat shock response is enhanced in juvenile compared with adult rainbow trout, *Oncorhynchus mykiss*. We have shown that the magnitude of the inducible heat shock response, as measured by increases in the heat shock protein (Hsp) Hsp70 is significantly greater in the hearts of fingerling than in adult trout following an acute (1 h) heat stress at 25 °C. Juvenile fish also express higher levels of constitutive Hsps (Hsc70, Hsp90) in their tissues, possibly reflecting higher rates of protein synthesis and increased chaperone function in this early life stage. This robust heat shock response in juvenile trout may contribute to a greater thermal resistance that is often observed in young salmonid fish.

Résumé : Nous concluons que la réaction au choc thermique est plus importante chez les truites arc-en-ciel, *Oncorhynchus mykiss*, juvéniles que chez les adultes. Nous avons montré que l'intensité de la réaction induite par le choc thermique, mesurée par l'augmentation de la protéine du choc thermique (Hsp) Hsp70, est significativement plus élevée dans le cœur des fretins que dans celui des truites adultes suivant un stress thermique aigu (1 h) à 25 °C. Les jeunes poissons ont aussi une expression de concentrations plus élevées d'Hsp constitutives (Hsc70, Hsp90) dans leurs tissus, ce qui reflète probablement des taux de synthèse de protéines plus élevés et une fonction de chaperon plus importante à ce stage précoce de leur cycle biologique. Cette réaction robuste au choc thermique chez les jeunes truites peut contribuer à la résistance thermique plus élevée que l'on observe souvent chez les jeunes salmonidés.

[Traduit par la Rédaction]

Introduction

Stream temperature is an important factor determining the abundance and distribution of cold-water fish species (de la Hoz Franco and Budy 2005). Although salmonid fish, like rainbow trout (*Oncorhynchus mykiss*), are considered cold-water fish, there are many examples of their persistence in warm water (>25 °C) environments (Ebersole et al. 2001; Rodnick et al. 2004; Spina 2007). Their presence in these apparently inhospitable conditions may be partially explained by behavioural thermoregulation whereby fish will seek out cooler pools and microhabitats (if available) to keep their body temperatures low (Torgersen et al. 1999). Prolonged warm temperatures in the summer months may not only decrease the size and (or) frequency of these thermal refugia, but also elevate metabolic demands and ultimately result in mortality (Brett 1979).

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Another factor that may reduce a fish's susceptibility to warm temperatures is the ability to protect their cells from heat-induced damage through the induction of heat shock proteins (Hsps). Hsps have been found in nearly every species examined to date, yet recent evidence suggests that these proteins are more important in the survival of ectotherms than endotherms (Shabtay and Arad 2005). Because body temperatures of ectothermic animals fluctuate with the environment, these organisms may have a greater need for Hsps compared with organisms with a more stable body temperature. Many fish species induce Hsps upon exposure to heat stress (e.g., Hightower et al. 1999; Currie et al. 2000; Fangue et al. 2006), presumably to protect and repair damaged proteins. Fish also possess high constitutive levels of Hsps within their cells and tissues (Currie and Tufts 1997; Boone and Vijayan 2002), pointing to important roles for these proteins in normal growth and development.

It is well established that the heat shock response decreases with age in many organisms. Most of this evidence comes from work on *Drosophila* (Niedzwiecki et al. 1991; Sørensen and Loeschcke 2002) and mammals (Heydari et al. 1993; Locke and Tanguay 1996; reviewed in Söti and Csermely 2000). Given the protective role of Hsps in the cell during stress, this decline is clearly a concern for aging ectotherms exposed to warming temperatures, as is the case for many fish species. There has been considerable work done on the heat shock response in developing zebrafish (*Danio rerio*) demonstrating that Hsps are both developmentally and spatially regulated (Krone et al. 1997; Lele et al.

1999; Sass et al. 1999). Further, Murtha and Keller (2003) found that aging modifies *hsp70* gene expression in zebrafish, as it does in other animals, in that basal and induced levels of *hsp70* mRNA were greater in juvenile versus mature fish. Effects of aging on Hsp expression have also been observed in vitro in nucleated red blood cells of rainbow trout where the heat shock response was attenuated in older cells (Lund et al. 2000). We have previously found that juvenile and adult trout respond differently to an acute thermal stress in terms of their intracellular distribution of Hsps (Rendell et al. 2006), but the overall capacity and magnitude of the heat shock response with age has not been examined. If Hsp expression is attenuated in aged fish after a thermal stress, as we hypothesize, this could increase the susceptibility of older fish to high temperature and possibly resultant infection or disease. Indeed, resistance to thermal stress decreases with age in many animals (Söti and Csermely 2000; Sørensen and Loeschcke 2002).

Climate warming and the continued human modification of land use (e.g., logging, urbanization, construction of dams) will increase temperatures in salmonid-producing streams (Schindler 2001), demanding a clearer understanding of the cellular implications of thermal stress. Thus, the goal of our work was to compare the magnitude of the heat shock response in juvenile and adult rainbow trout following an acute heat stress. Overall protein synthesis decreases with age in trout as they develop from fingerlings to adults, and this decline is tissue-specific (Peragón et al. 1998, 2001). We therefore decided to compare Hsp levels in several tissues of both fingerling and adult fish. Following heat shock, we have shown that the magnitude of Hsp induction (Hsp70) was significantly greater in the hearts of juvenile compared with adult rainbow trout and that overall, juvenile fish expressed higher levels of constitutive Hsps (Hsc70, Hsp90) than adults. This enhanced heat shock response may contribute to a greater thermal resistance in juvenile fish.

Materials and methods

Experimental animals

Rainbow trout fingerlings (mean mass = 1.65 g) were purchased from Ocean Trout Farms Ltd., Brookvale, Prince Edward Island, in June 2005. Adult rainbow trout (200–300 g, age 2+ years) were purchased from the Cardigan Fish Hatchery, Prince Edward Island, in May 2003 and were of the same genetic stock as the Brookvale fingerlings. Fingerling and adult fish were held for 4 and 29 months, respectively, prior to experimentation in 1 and 4 m^3 aquaria, respectively, each equipped with aerated, flow-through well water at 13 ± 1 °C. The photoperiod was a 16 h light:8 h dark cycle, and fish were fed to satiation every other day (floating salmonid feed; Corey Aquafeeds, New Brunswick, Canada). Feeding was terminated 24 h before each sampling period and resumed following recovery. All experimental procedures described here comply with the requirements outlined by the Canadian Council of Animal Care and were approved by the Mount Allison University Animal Care Committee (protocol No. 01–27).

Experimental heat shock protocol

Fish were transferred directly from their holding tanks into an aerated experimental tank (1 m × 0.7 m × 0.3 m) supplied with 13 ± 1 °C fresh well water 24 h before heat shock experiments to minimize handling stress. The heat shock protocol consisted of raising the temperature of the experimental tank from 13 to 25 °C by 3 °C per hour, holding the temperature at 25 °C for a 1 h heat shock. Following this heat shock, the water temperature was brought back down to 13 °C within approximately 4–6 h and remained at this temperature for the 8–24 h recovery periods (see below). We have previously determined that this heat shock protocol will consistently induce Hsps in 13 °C acclimated rainbow trout (Rendell et al. 2006; Currie et al. 2008), and the heat shock temperature of 25 °C is within the summer temperature range of what trout would experience in their native streams (Rodnick et al. 2004; Spina 2007).

Sampling

Six fish each were lethally anaesthetized in an aerated tank containing a buffered MS-222 (tricaine methanesulfonate) solution at four time points: control (prior to heat shock at 13 °C; 24 h following transfer to experimental tank), immediately following the 1 h heat shock (T = 0 h), and 8 and 24 h following the 1 h heat shock (T = 8 h and T = 24 h, respectively). White muscle, red muscle, liver, heart, and gill tissues were dissected and immediately freeze-clamped in liquid nitrogen for storage at –80 °C until further processing and analysis.

Protein extraction and quantification

Frozen tissue pieces were ground in a mortar and pestle under liquid nitrogen and weighed to the nearest 0.1 mg. Homogenization buffer (50 mmol·L^{-1} Tris, 2% sodium dodecyl sulphate (SDS), 0.1 mmol·L^{-1} protease inhibitor cocktail, Sigma, pH 7.5) was added at 15 µL·mg^{-1} of tissue, and samples were homogenized with a PowerGen 125 Polytron (Fisher Scientific). The homogenate was pushed through a 27 gauge needle to shear DNA and was centrifuged at 14 000*g* for 10 min in a Sorval Legend RT microcentrifuge at 4 °C to extract soluble protein. Supernatants were assayed for total protein concentration using the Bio-Rad DC Protein Assay for microtiter plates based on the Lowry method. Protein samples were diluted 1:10 in homogenization buffer (50 mmol·L^{-1} Tris, 2% SDS, 0.1 mmol·L^{-1} protease inhibitor cocktail, Sigma, pH 7.5), and total protein concentration was interpolated from a bovine serum albumin (BSA) standard curve read at 750 nm on a VERSAmax Tunable Microplate Reader (Molecular Devices Corporation).

Hsp immunodetection

Samples were prepared for SDS–PAGE with equal total protein loads of 15 µg in 2 × SDS reducing buffer (50% of total volume, 0.5 mmol·L^{-1} Tris–HCl, pH 6.8, glycerol, 10% SDS, 0.5% bromophenol blue) and 50 mmol·L^{-1} dithiothreitol (DTT). To compare protein levels among gels, a 15 µg liver protein sample from a heat-shocked trout (24 h after recovery) was selected as an internal standard for the Hsp70 gels. Recombinant human Hsp90 (Stressgen, SPP-770) and recombinant bovine Hsc70 (Stressgen, SPP-751) protein standards were loaded at 150 ng. Samples and standards were heated at 95 °C for 4 min and resolved in 10% Tris–HCl Pre-Cast Criterion gels (Bio-Rad).

Immunodetection proceeded as in Rendell et al. (2006). Rabbit antisalmonid Hsp90 (AS05 063), inducible Hsp70 (AS05 061), and constitutive Hsc70 (AS05 062) polyclonal antibodies (Agrisera, Sweden) were used at final concentrations of 1:50 000 in 2% blocking buffer (2% ECL Advance Blocking Agent, Amersham Biosciences, in Tris-buffered saline – Tween). Notably, the Hsp70 antibody is specific for the inducible isoform of Hsp70 and does not detect constitutive isoforms; the Hsc70 antibody detects the constitutive isoform and does not crossreact with the inducible Hsp70 (Rendell et al. 2006). Goat antirabbit IgG HRP conjugated secondary antibody (Stressgen, SAB-300) was used at 1:50 000.

Secondary antibody was detected using the ECL Advance Western Blotting Detection Kit (Amersham Biosciences). Protein bands were visualized using a FluorSMax MultImager High Sensitivity Digital Light Capture Camera (Bio-Rad). Experimental band densities were quantified using Quantity One software (Bio-Rad) and divided by the standard sample band density (heat-shocked trout liver or commercial standard) to give relative band density.

Statistics

All analyses were conducted using SPSS version 13 (SPSS Inc., Chicago, Illinois). Prior to analysis, data were checked for violation of assumptions of parametric tests. Normality was assessed visually using residuals and homogeneity of variance using Cochran's test. When variance heterogeneity was detected, the appropriate transformation was identified using Taylor's Power Law (Elliot 1977). Transformations were applied as follows: Hsp90 – $\log_{10}(x)$; Hsp70 – $x^{0.37}$; Hsc70 – $x^{-0.3}$; total protein content did not require transformation. In most cases, transformation corrected violations, and those that remained were minor, so analysis proceeded using parametric methods. In all cases, results were evaluated for significance at $\alpha = 0.05$.

Data were analysed using three-factor fixed-effect analyses of variance (ANOVAs), with treatment (heat shock; control, 0 h, 8 h, 24 h), development (adult, juvenile), and tissue (heart, liver, gill, white muscle, red muscle) as factors of interest. Response variables were Hsp90, Hsp70, Hsc70, and total soluble protein content. Because not all animals were assayed for each protein, it was necessary to conduct separate analyses for each response variable. When significant interactions were detected, data were split into levels of one of the interacting variables (typically tissue), and separate two-way ANOVAs were run (Keppel 1982). These analyses were preplanned, so α level corrections were not necessary. When main effects were detected, a posteriori comparisons were conducted using Tukey's test.

Results

Effects of heat shock treatment on total protein concentration varied across fish developmental stage and tissue (ANOVA developmental stage × treatment interaction, $p = 0.023$; developmental stage × tissue type, $p < 0.0001$). When effects of heat shock were examined separately for each tissue, we found that the heat shock resulted in significant increases in total soluble protein in the heart, gills, and red muscle that were maintained for 24 h into recovery (Fig. 1). Following heat shock, there were no increases in total soluble protein in the white muscle, and effects varied with developmental stage in the liver (ANOVA developmental stage × treatment interaction, $p = 0.017$). Total liver protein concentration increased with heat shock in juvenile fish ($p < 0.0001$), but not in adults ($p = 0.824$). Overall, adult fish tissues contained more total soluble protein than juvenile fish in the heart ($p < 0.0001$), gills ($p = 0.013$), and red muscle ($p = 0.032$). No developmental differences were observed for total protein in white muscle ($p = 0.297$), and differences in the liver could not be interpreted because of the significant developmental stage × treatment interaction (Fig. 1).

The magnitude of Hsp70 induction following heat shock varied with tissue type (ANOVA treatment × tissue type interaction, $p < 0.002$). However, when tissues were examined separately, there were marked treatment effects that were consistent between adults and juveniles ($p < 0.0001$ for every tissue). In all cases, controls were significantly lower than time points after heat shock, and in all tissues except gills, Hsp70 levels were greater at 8 and 24 h into recovery compared with immediately after heat shock, indicating that expression increased until 8 h, then remained elevated to the same level at 24 h (Fig. 2). In gills, there was no significant increase in Hsp70 levels with recovery time. The only significant developmental effect observed for Hsp70 was in heart tissue, where juvenile fish expressed significantly more Hsp70 than adult fish throughout the experiment ($p = 0.001$; Fig. 2).

Effects of heat shock on Hsc70 levels varied with tissue type (ANOVA treatment × tissue interaction, $p = 0.048$), as did differences between developmental stages (ANOVA development × tissue interaction, $p < 0.0001$). Hsc70 expression levels varied with time after heat shock in the gills ($p = 0.046$) and the white muscle ($p = 0.04$) only, but there were no consistent trends over time (Fig. 3), and a posteriori testing failed to reveal any significant pairwise differences. Juvenile fish expressed more Hsc70 in the white muscle and red muscle ($p < 0.0001$), whereas adult fish expressed more Hsc70 in the liver ($p = 0.018$). Gill and heart tissues showed no significant developmental differences in Hsc70 expression ($p = 0.412$ and 0.76, respectively) (Fig. 3).

Hsp90 levels were significantly elevated in juveniles relative to adults ($p < 0.0001$) and were higher in heart, gill, and liver tissues than in both muscle types ($p < 0.0001$ and post hoc testing; Fig. 4). Heat shock did not alter the expression of this protein in the tissues of adult or juvenile fish ($p = 0.49$).

Discussion

We hypothesized that the induction of Hsps with heat shock would be greater in juvenile fish compared with their adult counterparts, which may partially explain why juvenile fish are more resistant to thermal stress than older fish. Rainbow trout prefer temperatures anywhere from ~10 to 22 °C, with a lethal thermal maximum as high as 32 °C (see Spina 2007 for review). In the work presented here, we examined changes in the expression of three Hsps in rainbow trout from two different development stages and in

Fig. 1. Total soluble protein concentration per gram (mg·g^{-1}) of heart (*a*), liver (*b*), gill (*c*), white muscle (WM, *d*), and red muscle (RM, *e*) tissues in adult (▲) and juvenile (■) (*Oncorhynchus mykiss*) sampled prior to heat shock (control at 13 °C) and at 0, 8, and 24 h following heat shock (at 13 °C). Data points represent replicate means ± standard error of the mean (SEM), and n = 5–6 for all groups. An asterisk (*) indicates a significant difference from control in adults or juveniles, and different letters indicate an overall significant difference between adults and juveniles. The vertical broken line represents the 1 h heat shock.

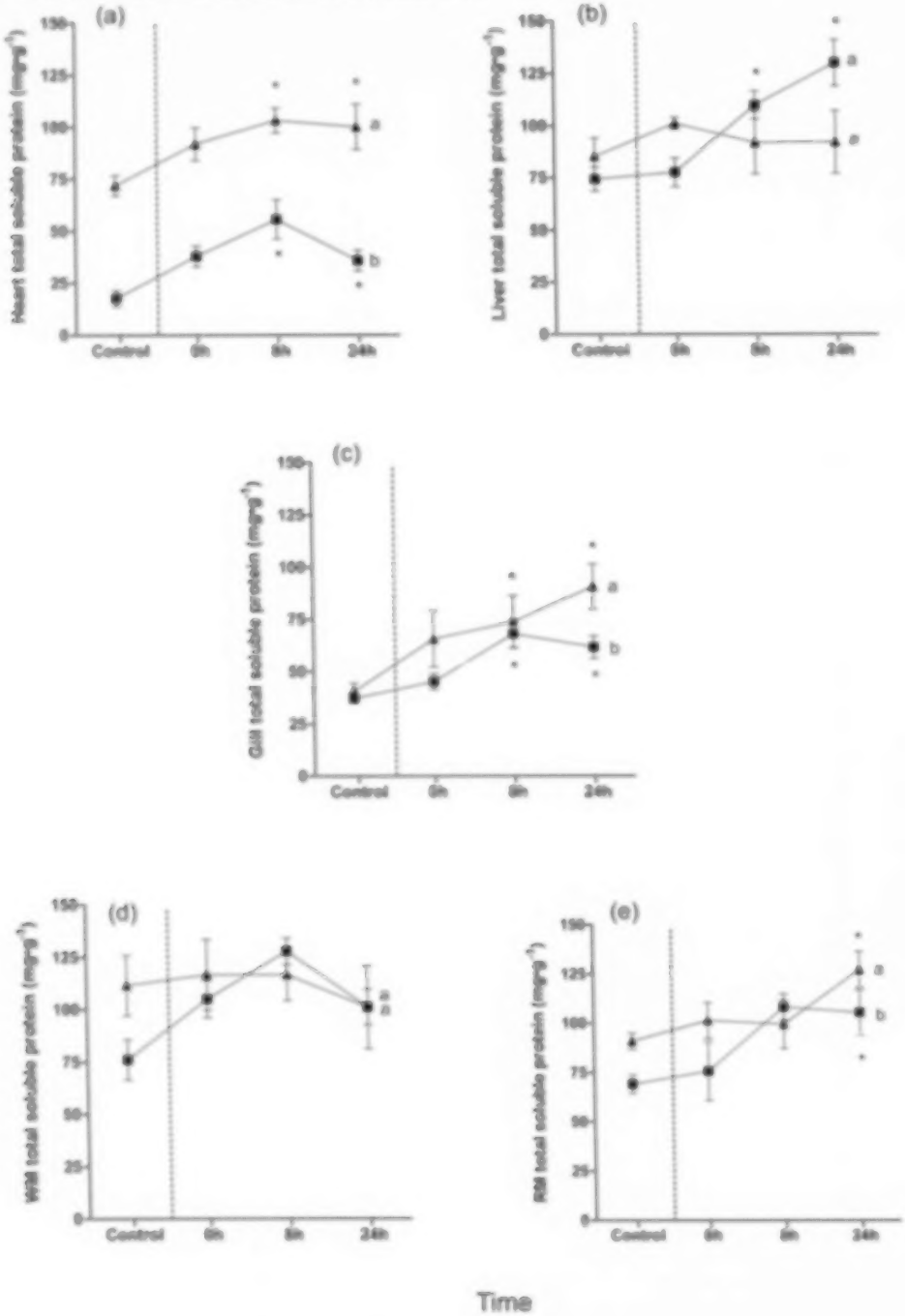


Fig. 2. Hsp70 relative band density in heart (*a*), liver (*b*), gill (*c*), white muscle (WM, *d*), and red muscle (RM, *e*) tissues in adult (▲) and juvenile (■) fish sampled prior to heat shock (control at 13 °C) and at 0, 8, and 24 h following heat shock (at 13 °C). Data points represent replicate means ± standard error of the mean (SEM), and $n = 4$–6 for all groups except 8 h red muscle, where $n = 3$ (due to loss of samples in –80 °C freezer). An asterisk (*) indicates a significant difference from control in adults or juveniles, and different letters indicate an overall significant difference between adults and juveniles. The vertical broken line represents the 1 h heat shock.

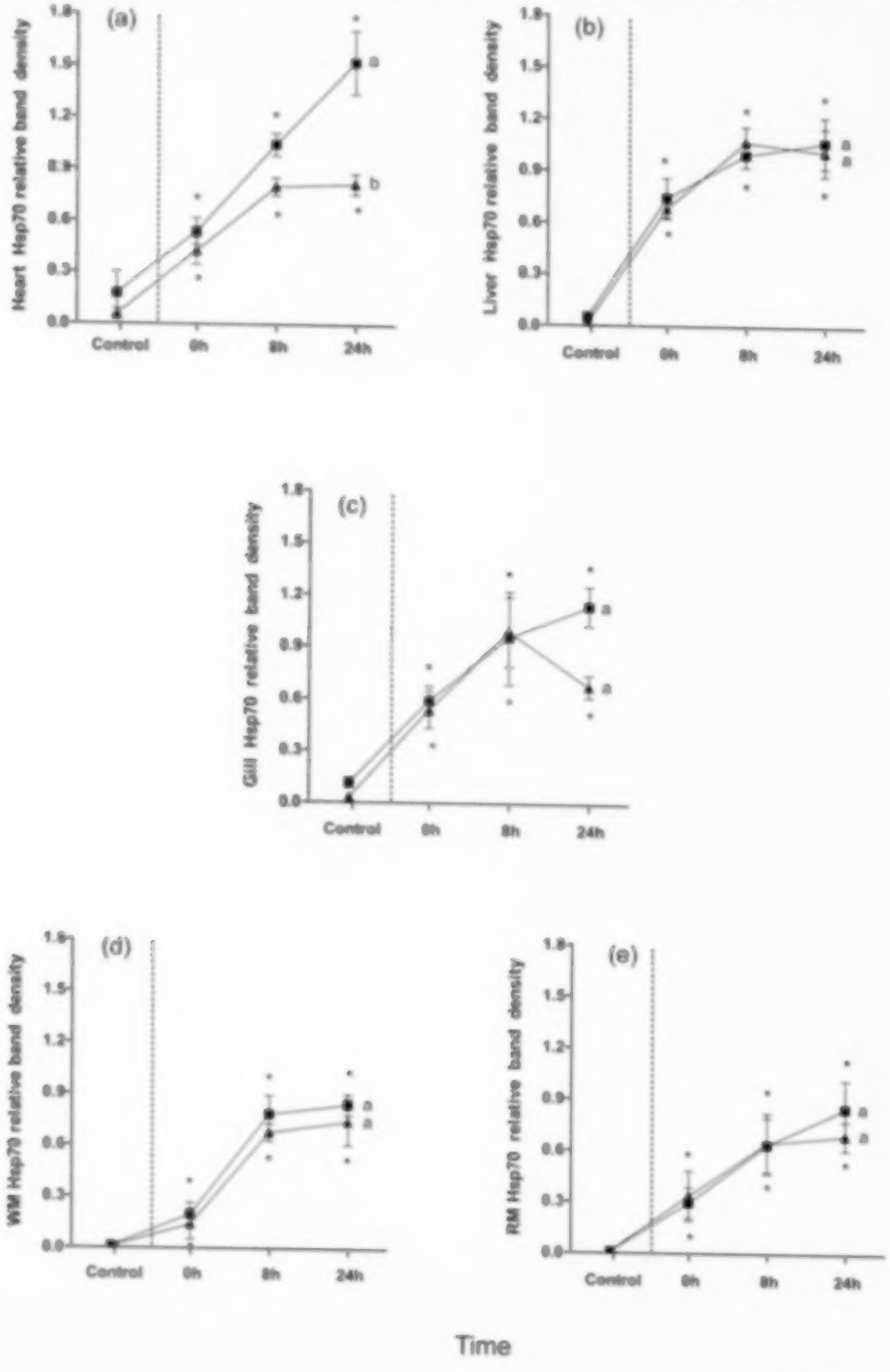
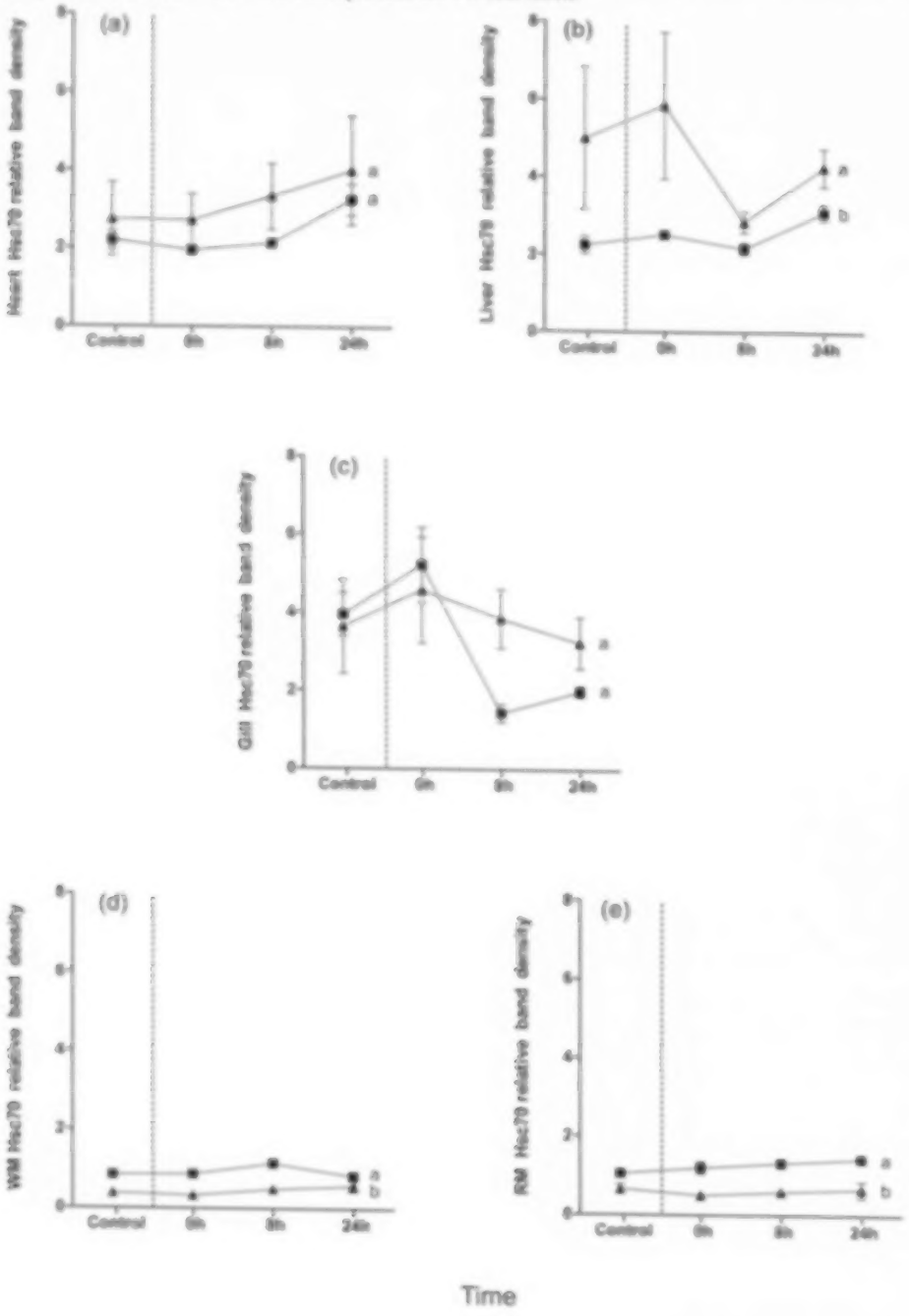


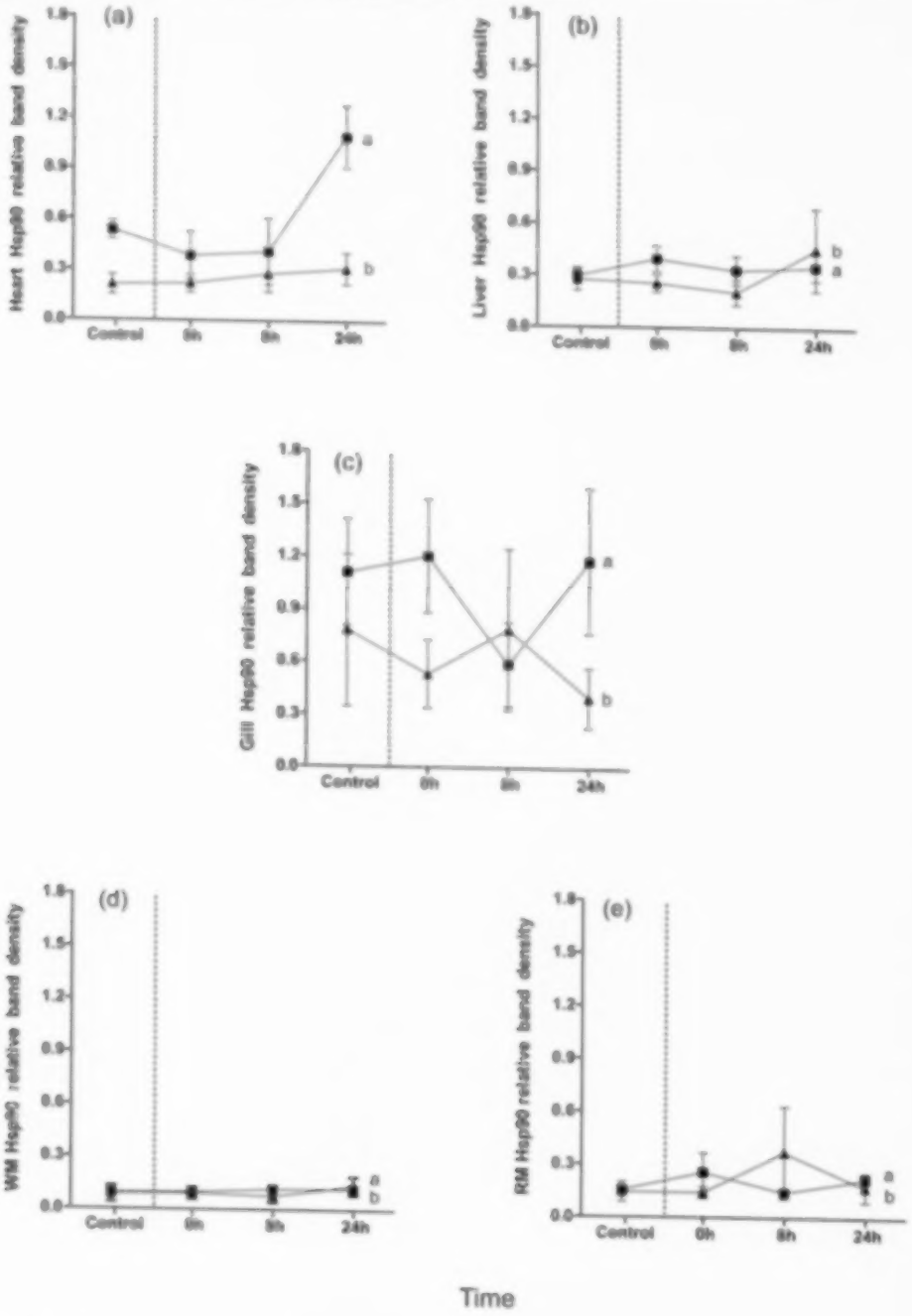
Fig. 3. Hsc70 relative band density in heart (*a*), liver (*b*), gill (*c*), white muscle (WM, *d*), and red muscle (RM, *e*) tissues in adult (▲) and juvenile (■) fish sampled prior to heat shock (control at 13 °C) and at 0, 8, and 24 h following heat shock (at 13 °C). Data points represent replicate means ± standard error of the mean (SEM), and $n = 5$–6 for all groups. Different letters indicate an overall significant difference between adults and juvenile. The vertical broken line represents the 1 h heat shock.



five tissues before and after exposure to acute heat stress. We observed tissue- and protein-specific induction profiles, but overall, juvenile tissues expressed higher levels of Hsps.

It is now fairly well established that susceptibility to heat stress increases as animals age. This phenomenon has been well described in *Drosophila* (Niedzwiecki et al. 1991; Ser-

Fig. 4. Hsp90 relative band density in heart (*a*), liver (*b*), gill (*c*), white muscle (WM, *d*), and red muscle (RM, *e*) tissues in adult (▲) and juvenile (■) fish sampled prior to heat shock (control at 13 °C) and at 0, 8, and 24 h following heat shock (at 13 °C). Data points represent replicate means ± standard error of the mean (SEM), and $n = 4–6$ for all groups. Different letters indicate an overall significant difference between adults and juveniles. The vertical broken line represents the 1 h heat shock.



ensen and Loeschcke 2002), and there is evidence that this is also the case in mammals (Söti and Csermely 2000) and in fish. In early work on salmonids (brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*)), upper thermal tolerances in parr were 2–4 °C greater than in alevins (Spaas 1960). More recently, Rodnick et al. (2004) reported that the

critical thermal maximum temperatures of juvenile and adult redband rainbow trout (*Oncorhynchus mykiss*) were similar. However, they observed that larger, older trout were more thermally sensitive and incurred higher metabolic costs than smaller, younger trout when acutely exposed to high water temperatures. Indeed, young fish appear to prefer warmer temperatures and will select higher temperatures than their older conspecifics (McCauley and Huggins 1979).

One explanation for this decreased heat resistance with age in animals is the decreased expression of Hsps (Söti and Csermely 2000). In *Drosophila*, heat-induced Hsp expression decreases with age closely coinciding with a decreased heat resistance (Sørensen and Loeschcke 2002). Age differences also exist in the heat shock response of zebrafish. Basal and heat-induced levels of *hsp70* gene transcription were reduced in adult versus young zebrafish (Murtha and Keller 2003). In adult and juvenile rainbow trout exposed to metal contaminants in water and food, only juvenile fish induced Hsp70 (Williams et al. 1996). This decrease in the heat shock response with age has also been shown at the cellular level in rainbow trout. Older red blood cells have a significantly reduced capacity to induce Hsps compared with younger cells (Lund et al. 2000), and it is thought that the cells lose the ability to detect the stress and (or) respond appropriately with increased chaperone expression; either situation would render the cell and organism more susceptible to stress.

In our experiments, Hsp70 was dramatically induced in all tissues with heat shock, but heart was the only tissue where we observed developmental differences, with adults showing a significantly lower induction of Hsp70 than juveniles. Locke and Tanguay (1996) also observed a reduced Hsp72 response following heat shock in the hearts of aged rats. They noted that the decreased ability of a vital organ, like the heart, to withstand heat stress (with reduced Hsp expression) might increase the stress susceptibility of the whole animal. We did not observe any age-related changes in the other trout tissues examined. However, with regard to muscle tissue, it is noteworthy that the lack of developmental differences in Hsp70 is also supported by work on zebrafish. *hsp70* gene expression differences were absent in the muscles of young and old fish, despite differences in *hsp70* genes with age in the brain, another vital tissue (Murtha and Keller 2003). Thus, a more robust induction of Hsps in vital tissues (e.g., heart and brain) of juvenile fish may be a mechanism explaining greater stress resistance in younger fish.

In contrast with the inducible protein Hsp70, the expression of its constitutive isoform Hsc70 did not change during heat stress; however, we did observe distinct age- and tissue-specific Hsc70 profiles. Compared with juvenile fish, levels of Hsc70 were significantly higher in the liver and significantly lower in the muscle tissue of adult fish. These contrasting results could be explained by the level of protein damage incurred in each tissue. Because aging results in a loss of adaptive responses to stress, it may increase proteotoxic conditions within cells (Söti and Csermely 2000). This means that damaged, misfolded proteins would accumulate in aged animals, thus requiring increased levels of basal, constitutive chaperones, like Hsc70, to assist in proper protein folding, regardless of the stressed state of the cell. We observed higher liver Hsc70 levels in adults compared with juveniles, a finding supported by work on mice where liver *hsc70* gene expression also increased with age (Dhahbi et al. 1997; Cao et al. 2001), possibly in response to increases in oxidative stress in this tissue, characteristic of the aging process. It is also noteworthy that liver tissue maintains the potential for regeneration as it ages and may require sustained chaperone action through senescence, compared with other tissues such as postmitotic skeletal muscle. Indeed, the heat shock response in trout is tissue-specific (Currie et al. 2000; Lund et al. 2003), and tissues may be subjected to distinct stressors during aging resulting in individual Hsp profiles (Cao et al. 2001).

To illustrate this point and in contrast with liver tissue, Hsc70 levels are significantly greater in the muscle (both red and white) of juvenile fish. Basal levels of Hsp70 were also higher in young zebrafish muscle compared with tissues from older animals (Murtha and Keller 2003). These tissue differences in constitutive Hsc70 may simply reflect the tissue-specific Hsp profiles with age in fish and (or) may be indicative of higher rates of whole-body protein synthesis in juvenile fish compared with adults (Peragón et al. 1998, 2001). These higher rates may be partly explained by increased food intake and growth rates in fingerling compared with adult trout (Peragón et al. 2001) and likely support a more active metabolism and the tissue demand for exported proteins characteristic of early life stages (Schmidt-Nielsen 1984; Peragón et al. 1998). Protein synthesis also declines dramatically in aging rainbow trout red blood cells, although protein content remains constant, possibly explained by an adult protein pool with long half-lives and lower turnover rates (Lund et al. 2000).

Our results for Hsp90 expression lend further support to the notion of higher rates of protein synthesis in juvenile fish. In all tissues examined, Hsp90 levels are significantly greater in juvenile compared with adult fish. In recent work from our laboratory, we also had indications that Hsp90 may be differentially expressed with age. We saw distinct Hsp90 subcellular localization profiles in juvenile and adult rainbow trout heart and liver tissues (Rendell and Currie 2005; Rendell et al. 2006). Unlike the stress-inducible Hsp70, Hsp90 is highly expressed constitutively under normal growth conditions and acts as an important chaperone in many cell-signalling systems (Pratt and Toft 1997). However, minor inductions of Hsp90 have been observed with heat shock in rainbow trout heart and liver (Rendell et al. 2006) and cultured hepatocytes (Sathiyaa et al. 2001). On the whole, the higher constitutive levels of Hsps seen in juvenile trout may reflect the higher growth rates, nutritional requirements, and protein turnover at this early developmental stage.

In summary, we have shown that the magnitude of the inducible heat shock response is significantly greater in the hearts of juvenile fish, possibly contributing to an enhanced stress tolerance compared with their adult counterparts. Although the Hsc70 expression profile is tissue-specific with age, juvenile trout tend to have higher levels of constitutive Hsps. This may reflect higher rates of protein synthesis and the need for the cell to chaperone newly synthesized proteins during periods of accelerated growth. For aging animals with a reduced heat shock response, they will likely be

more susceptible to cell damage when exposed to the stress of high water temperatures, a situation with clear fitness implications.

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Predicting the vertical distribution of the opossum shrimp, *Mysis relicta*, in Lake Ontario: a test of laboratory-based light preferences

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Abstract: Light and temperature strongly influence the vertical distribution of the mysid shrimp, *Mysis relicta*. We monitored the vertical movements and depth selection behavior of mysids exposed to different light intensities and light–temperature gradients in the laboratory and derived a mysid light preference function in units relevant to mysid vision: "mylux". Mysids preferred light levels between 10^{-6} and 10^{-7} mylux ($\sim 10^{-6}$ to 10^{-5} lux) and rarely moved into waters of 10^{-3} mylux (~ 0.1 lux) and greater. A model that assumed equal weight and independence of mysid light and temperature preference functions successfully predicted the proportion of mysids found in two different temperature–light combinations in the laboratory. This model also predicted the depth of maximum mysid density to within 2 m on two spring nights and within 5 m on two summer nights of varying moon phase and thermal conditions in Lake Ontario. This study provides novel insights into how temperature and light interact to influence the vertical distribution of mysids. Our model may be used to predict mysid vertical distribution in any deepwater system inhabited by mysids in which the primary mysid predators are visual feeders.

Résumé : La lumière et la température influencent fortement la répartition verticale des mysis, *Mysis relicta*. Nous avons noté les déplacements verticaux et le comportement de sélection de profondeur de mysis exposés à différentes intensités lumineuses et à des gradients de lumière–température en laboratoire et avons mis au point une fonction de préférence lumineuse des mysis en unités « mylux » qui sont compatibles avec la vision des mysis. Les mysis préfèrent des intensités lumineuses de 10^{-6} à 10^{-7} mylux ($\sim 10^{-6}$ à 10^{-5} lux) et pénètrent rarement dans des eaux de 10^{-3} mylux ($\sim 0{,}1$ lux) ou plus. Un modèle qui présume un poids égal et une indépendance des fonctions de préférence de lumière et de température des mysis prédit avec succès les proportions de mysis retrouvées dans deux combinaisons différentes de température–lumière en laboratoire. Ce modèle prédit aussi la profondeur de densité maximale des mysis avec une précision de 2 m lors de deux nuits de printemps et une précision de 5 m lors de deux nuits d'été de phase lunaire et de conditions thermiques différentes au lac Ontario. Notre étude ouvre des perspectives nouvelles sur l'influence de l'interaction de la température et de la lumière sur la répartition verticale des mysis. Notre modèle peut servir à prédire la répartition verticale des mysis dans n'importe quel système d'eau profonde habité par les mysis et dans lequel les principaux prédateurs des mysis se nourrissent à vue.

[Traduit par la Rédaction]

Introduction

Zooplankton diel vertical migration (DVM) is a widely occurring and well-documented phenomenon in both marine and freshwater ecosystems (Hamner 1988; Haney 1988). Although a vast literature exists on the different proximate cues (i.e., light, temperature, and chemical cues) influencing zooplankton DVM, there is still no general consensus as to how these different exogenous factors interact to impact a migrating population's vertical distribution. Light is generally considered to be the most important proximate factor influencing zooplankton DVM (Lampert 1991; see reviews by Forward 1988 and Ringelberg 1999); however, whether migrating organisms simply maintain a preferred light condition throughout their migration and night or day distribution (the preferendum, or isolume, hypothesis) is still an open question and may be species-dependent.

The opossum shrimp, *Mysis relicta* (or *Mysis diluviana* after Audzijonyte and Väinölä 2005; hereafter referred to as "mysids" unless otherwise noted), exhibits DVM in many deep, freshwater lakes of North America. Mysids are a highly nutritious food item for the planktivorous fish community but are also important predators of zooplankton in these systems, thereby acting as competitors with the planktivorous fish that eat them (Gal et al. 2006). The availability of mysids as prey to these fish, as well as the degree to which they contribute to pelagic zooplanktivory, is strongly influenced by light (see review by Beeton and Bowers 1982; Gal et al. 2004). For example, mysids are typically found deeper in the water column on full moon versus new moon nights (Janssen and Brandt 1980; Beeton and Bowers 1982). Similar observations of light deterrence have been recorded

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in other lakes containing mysids (Moen and Langeland 1989). Even dim boat lights and passing clouds can modify mysid vertical distribution in the water column at night (Gal et al. 1999; Johannsson et al. 2003). Since subtle changes in light can impact where mysids are found in the water column and therefore their overall contribution to the pelagic food web (Gal et al. 2004), the ability to model their response to light and predict their distributions based on ambient light conditions is important for both mysid ecology and food web dynamics in lakes where they occur.

While it is clear that mysids are sensitive to changing light levels, the precise intensities necessary to limit or enhance vertical movements are less understood. Teraguchi et al. (1975) reported that the upper edge of the *M. relicta* migratory layer in Green Lake, Wisconsin, was associated with a narrow light interval of 0.02–0.05 lux, while *Mysis mixta* in the Baltic Sea was shown to avoid light levels of 10^{-4} lux (Rudstam et al. 1989). However, lux is a unit of measure associated with luminosity and is specific for what the human eye perceives; therefore, the lux unit does not accurately convey the level of brightness perceived by a mysid eye. Gal et al. (1999) measured the absorbing pigments of mysids from Cayuga Lake, New York, using a microspectrophotometer and derived a mysid-specific brightness unit, the "mylux", which accounts for the relative sensitivity of the mysid eye to different wavelengths of light. Using spectral sensitivity curves to derive species-specific brightness units represents the most appropriate way to report the amount of light perceived by an individual organism (Jerlov 1963). In this study, we report all light levels in the biologically relevant units of mylux.

The degree to which mysids prefer a specific light intensity or range of intensities, as well as how this preference may vary between discrete populations, is also unknown. Gal et al. (2004) generated a mysid light preference function, using the mylux unit, based on acoustic data collected in the eastern portion of Lake Ontario in May 1996 (Gal et al. 2004). The preference curve indicated that mysids preferred 10^{-7} mylux, which in Lake Ontario waters on a cloudless, moonlit night is equivalent to $\sim 10^{-5}$ lux. However, the data used to generate the light preference curve were collected on the same lake and only one night later than the data used to test the model. Other factors such as chemical cues from predators and prey may affect mysid distribution (Boscarino et al. 2007) and would not have been accounted for in the derivation of the light preference curve. To our knowledge, there have been no studies that have explicitly investigated the responses of mysids to different light levels in the laboratory, to either verify or refute these field-derived light preferences.

Other factors besides light may also serve as controlling mechanisms of mysid vertical distribution. For example, temperature may become increasingly important in determining final depth preferences when the lake is thermally stratified during the summer and fall seasons (Gal et al. 2004; Boscarino et al. 2007). Recently, Boscarino et al. (2007) developed a temperature preference curve, with a peak between 6 and 8 °C, based on observations of mysid movements in thermally stratified experimental columns. These authors hypothesized that mysids developed a preference for such a narrow range of relatively low temperatures in response to high predation pressure in shallow waters or to maximize growth in the strata just below the thermocline (Boscarino et al. 2007). However, mysid temperature preference may be modified by light level; similarly, mysid light preferences may vary with temperature. The interaction between temperature and light has yet to be tested under controlled conditions, and therefore the relative importance of light and temperature in determining mysid vertical distribution is not known. In our previous models, light and temperature preference functions were assumed to have equal weight and be independent (Gal et al. 2004; Boscarino et al. 2007).

In this study, we test the hypothesis that mysids prefer certain light intensities and will avoid others. Our approach was to monitor the behavioral responses of mysids to different manipulations of light in 2 m tall observation columns in the laboratory. We use the results of these light preference experiments to construct a function describing the relative probability of observing a mysid at different light levels and combine this light function with a temperature preference function to yield a predictive model of mysid vertical distribution (e.g., Boscarino et al. 2007). We test the ability of this model to predict mysid vertical distribution by comparing model predictions with observed mysid distributions under different light–temperature combinations in our experimental columns. This procedure tests the assumption of Gal et al. (2004) and Boscarino et al. (2007) that temperature and light preference functions are equally important and independent predictors of mysid vertical distribution. We also use this model to predict published field distributions of mysids in Lake Ontario during the spring when light is hypothesized to limit mysid vertical movements and during the summer when temperature and light both influence distribution (see discussion in Johannsson et al. 2003).

Materials and methods

Experimental outline

We conducted three experiments to determine the effects of light on mysid vertical movement and depth selection behavior as well as investigate the interaction between light and temperature preferences. First, we observed and quantified mysid preferences for different light levels (light experiment). We then used these results and the temperature preferences derived by Boscarino et al. (2007) to evaluate the assumption of Gal et al. (2004) and Boscarino et al. (2007) that temperature and light preference functions are equally important and independent predictors of mysid vertical distribution. To test this assumption, we observed and quantified the distribution of mysids under two light–temperature combinations in our experimental columns: (*i*) the deterring light – preferred temperature (DLPT) combination experiment in which a preferred temperature (6 °C) was combined with a light level known to elicit an avoidance response (10^{-2} mylux) and (*ii*) the preferred light – limiting temperature (PLLT) combination experiment when a preferred light condition (10^{-6} mylux) was combined with a temperature known to limit mysid vertical ascent (12 °C) (Boscarino et al. 2007).

Collection and maintenance of mysids

Mysids used in experiments were collected with vertical

net hauls (1 m diameter, 1 mm mesh) on a new moon night in October 2005 at a 100 m deep site in Skaneateles Lake, New York. Skaneateles Lake is a deep, oligotrophic lake in the Finger Lakes region of New York State that drains northward into Lake Ontario. The spectral sensitivity curves of both the Skaneateles Lake and Lake Ontario mysids are very similar and are both best fit by a vitamin A_1 template curve (B. Boscarino, unpublished data). Mysids were placed into 4 °C, light-proofed coolers immediately following collection to avoid extended exposure to adverse light and thermal conditions. Mysids were fed ad libitum rations of Cyclop-eez, a food source derived from the subclass Copepoda, on a daily basis. Those mysids selected for use in the experiments were starved for approximately 12 h prior to experimentation to ensure that they would be active when they entered the experiment. Only adult and subadult mysids (>12 mm) were selected for use in this experiment (mean length = 14 mm), and these were selected at random (male or female, although no ovigerous females were used) from our stock tanks. Mysids were euthanized and measured immediately following the experimental trial.

All feeding and handling of mysids were conducted under infrared or near-infrared conditions, as mysids are insensitive to these wavelengths of light (Jokela-Määttä et al. 2005). An opaque blind was placed immediately outside the entrance door of the experimental room to prevent fluorescent light from entering. In addition, black felt was hung on all four walls of the experimental room to prevent light from reflecting off the walls onto the experimental columns.

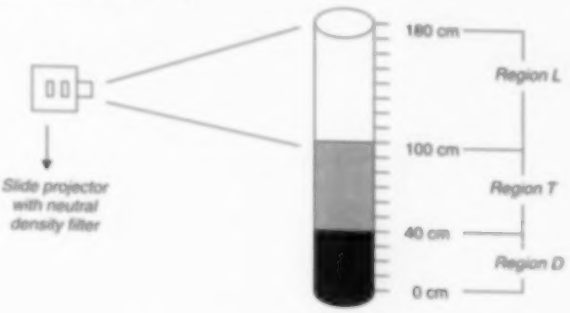
Experimental columns

The setup of the observational columns was described by Boscarino et al. (2007). Briefly, experimental columns were 2 m tall Plexiglas cylinders and held approximately 8 L of dechlorinated, Lake Ontario water (Fig. 1). Water temperatures were maintained at 4 °C by the temperature of the room. Columns were labeled from 0 cm (bottom of column) to 180 cm (top of column) for mysid depth reference during behavioral observations (Fig. 1). Thermal gradients in the DLPT combination and PLLT combination experiments were created by lowering a heater down to the 90–110 cm interval of the column and controlling the temperature of the upper portion of the water column by setting an Aqualogic digital temperature controller to the desired temperature. Temperature varied <1 °C throughout an entire 45 min time interval at each depth that temperature was recorded.

Establishment and measurement of light gradients

The light source for the three experiments was a slide projector (Kodak Carousel 5200), which was equipped with a Wiko 120V, 300W ELH light bulb. The projector was placed approximately 4 m away from the columns and projected light onto the upper portion of the experimental columns (Fig. 1). Intensity was controlled by placing a set of neutral density filters (Kodak Wratten gelatin neutral density filters) in the projector. We used different combinations of these neutral density filters (D = 1.0–4.0) to achieve the desired light intensity reaching the top portion (i.e., 100–180 cm) of the experimental columns. Light was prevented from illuminating the bottom portion of the columns (0–40 cm) by placing opaque tape on a portion of a neutral density slide as well as placing an opaque board, which extended up to the 40 cm line, a few metres away from the column. Each combination of a dark bottom column (region D) and illuminated upper column (region L) was considered a treatment. Replicates of each treatment were considered experimental trials. For each treatment, there was also a transition region between the completely dark region D (0–40 cm) and the lighted region L (100–180 cm), which we will hereafter refer to as the transition region, region T (40–100 cm). Region T began when the photometer registered a light level greater than zero and ended when the desired region L intensity had been reached. Region depth designations remained consistent across all experimental treatments (Fig. 1).

Fig. 1. Schematic of experimental setup. Region L represents the portion of the column illuminated with the treatment intensity (100–180 cm), while region D (0–40 cm) is completely dark. Region T (40–100 cm) is the transition region between the desired illumination in region L and complete darkness. Regions are shaded to reflect general differences in light intensities and are not to scale of color. Neutral density filters are inserted into the projector to control overall intensity.



We recorded overall radiance with an International Light light meter (model IL1400A), which had a 5° acceptance angle and was calibrated to a Gamma Scientific light source. The light meter has an accuracy of ±2% of the International Light calibration transfer standards. The light meter was also fitted with a "mysid filter" (Rosco Roscolux filter, #91, peak of filter = 510 nm) selected such that the detector–filter combination closely matched the spectral sensitivity curve of a mysid visual pigment (e.g., Widder and Frank 2001). Thus, this light meter measures radiance relevant to mysid vision (Gal et al. 1999). We chose to measure light levels in terms of radiance rather than irradiance because radiance is a more biologically relevant measure of the amount of light reaching a photoreceptor in a mysid eye than irradiance (e.g., Loew and McFarland 1990). Each photoreceptor in a mysid eye has a limited acceptance angle in which to receive incoming light and therefore does not follow the cosine characteristics associated with measures of irradiance. Therefore, a mysid eye acts more like a radiance detector than an irradiance detector. However, we converted all radiance measurements obtained in the laboratory to the irradiance-based mylux unit for ease of comparisons with the literature, where light intensity is typically reported in terms of irradiance.

To determine these radiance to irradiance conversions, we measured the total available light during four different

nights of varying moon phase (ranging from new moon to full moon) in 2005–2006 with a calibrated archival tag (mk9 Wildlife Computers, Redmond, Washington, USA). This mk9 tag was fitted with the same mysid filter, had the same acceptance angle as that of the International Light meter used in the Light experiments, and was calibrated with the same standard. Therefore, both the mk9 tag and the light meter measured radiance in the same mysid-specific unit. The advantage to using this archival tag versus our International Light meter directly is that we were able to record light readings at 4 min intervals throughout each of these four nights and were therefore able to track changes in light due to moon altitude, as well as phase. The International Light meter did not have these data-logging capabilities. For each time interval in which we sampled with the mk9 tag, we retrieved the corresponding value of moonlight illuminance, in lux, from the computer program of Janiczek and DeYoung (1987). Since the Janiczek and DeYoung model is not capable of predicting light levels at new moon, we used values derived by the moonlight illuminance model of Austin et al. (1976) for new moon nights and nights in which the moon was below the horizon. These two models give similar moon illuminance values when zenith angles are approximately the same. We converted these predicted model values in lux to mylux using the conversions of Gal et al. (1999): 1 mylux = 175 lux = 0.51 W·m^{-2}. For each 4 min time interval, we calculated the ratio between the measured radiance with the mk9 tag (i.e., mk9 units) and the predicted irradiance in mylux based on the Janiczek and DeYoung and Austin et al. (1976) models. This ratio was 30.0 mk9 units:1 mylux (standard error (SE) of ratio = 1.8; n = 106). We converted all mk9 and International Light meter readings directly into mylux based on this ratio. All light levels in this study are therefore reported in the same mylux values presented in Gal et al. (1999, 2004) and Boscarino et al. (2007).

Note that the conversions between lux, W·m^{-2}, and mylux first presented in Gal et al. (1999) will change with depth, since the spectral distribution of light changes with depth because of differences in wavelength-specific attenuation. For example, given the measured k_{PAR} and associated wavelength-specific attenuation from Jerome et al. (1983), the ratio of lux to mylux increased from 175:1 at the surface to 192:1 at 50 m depth on the two spring nights and decreased from 175:1 to 20:1 on the two August nights used to test our vertical distribution model. Therefore, all conversions between mylux and lux below the surface are approximate and should be used only as a rough estimate for comparison with other studies that report light in lux rather than more appropriate, species-specific units.

Experiments

Behavioural observations

Three mysids were placed into the bottom portion of the observation column for every trial. We did not start recording mysid position in the water column until at least 6 min after the start of the experiment; this time is needed for the mysids to become randomly distributed in the column during isothermal dark conditions (Boscarino et al. 2007). After this 6 min acclimation period, mysid positions (to the nearest 10 cm) were recorded every 3 min over a period of 45 min for a total of 15 observations per mysid using an infrared, digital video camera recorder (Sony Digital Handy-Cam, model TRV18).

Experiment 1: light experiment

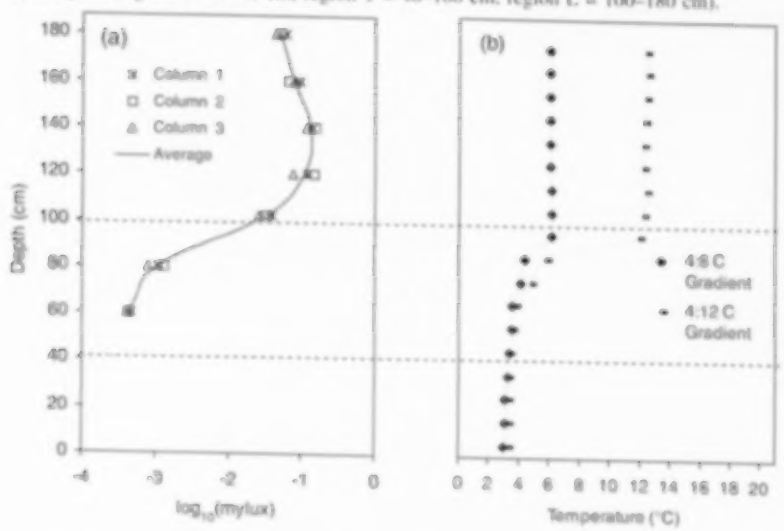
Three columns were used to monitor mysid movements. Each column was held uniformly at 4 °C. One light treatment was administered to all three columns simultaneously, and we noted no major changes in water temperature associated with the illumination of the columns. Light intensity was recorded with the mysid-specific photometer at 10 cm depth intervals throughout each column over a 45 min time period to determine the degree to which light intensity fluctuated over one trial period as well as to what degree intensity levels varied from column to column. The range in light levels varied less than a factor of two for each depth interval throughout the 45 min time period, and there was no significant difference in light levels at the same depth among columns (two-way analysis of variance, ANOVA, depth–column interaction effect, p = 0.98, n = 21).

One completely dark condition (control) and 10 light gradients were established in the light experiments to monitor the relative light preferences of mysids. Gradients will hereafter be expressed in region D:region L light ratios. Region L was varied exactly one order of magnitude for each experimental treatment, starting at 10^{-10} mylux and continuing up to 10^{-1} mylux (i.e., treatment 1 = dark: 10^{-10} mylux gradient; treatment 2 = dark: 10^{-9} mylux gradient, etc.). We did not administer a light level treatment any higher than 0.1 mylux, as these light levels approximate dawn and dusk light levels on Lake Ontario, and thus, mysids should not experience light levels any higher than this during nighttime migration (Gal et al. 1999).

The region L light intensity used to define each treatment was expressed as the mean light intensity experienced by a mysid in region L (100–180 cm). The range of light levels recorded in region L varied by less than a factor of five (Fig. 2*a*). The average light gradient experienced by a mysid in all subsequent treatments depended directly on the number of neutral density filters in front of the projector, as each filter decreased light levels by one log unit. The photometer did not detect any light in region D even at the highest light level treatment. Given that our photometer is sensitive to light levels of 10^{-10} mylux, light levels in region D were less than 10^{-10} mylux in all treatments. Control (completely dark) trials were conducted separately from the light treatment trials, since we could not ensure completely dark column conditions while using the projector to generate light gradients in the other columns.

The proportion of observations in region L relative to those in regions L and D in each column was considered an independent data point (Boscarino et al. 2007). Proportions were used as data points since individual observations of position within the water column may not have been independent. Region T observations were excluded from the analysis because region T light intensities were highly variable between treatments, so direct intertreatment comparisons were not possible. One-way ANOVA and Dunnett's t test (α = 0.05) were performed, after arcsine transformation of the proportions, to test for differences in the proportion of observations at different light intensities relative to control

Fig. 2. Experimental light and temperature gradients. (*a*) Base light gradient administered in the light experiments. Measurements represent the recorded light intensities, in $\log_{10}$(mylux), at 20 cm intervals in each of the three experimental columns at the highest light level treatment. All other region L intensity treatments were assumed to follow the same relative gradient, but were dark-shifted exactly one order of magnitude for every neutral density filter placed in the slide. No light intensities are plotted for depths below 40 cm (region D), because this portion of the column remained less than 10^{-10} mylux for all treatments. (*b*) Temperature gradients established in the deterring light – preferred temperature (DLPT) combination experiment and in the preferred light – limiting temperature (PLLT) combination experiment. Broken lines separate each light region (region D = 0–40 cm, region T = 40–100 cm, region L = 100–180 cm).



(completely dark) conditions in the light experiments. After arcsine transformation, we noted no trends in the residuals when regressed on predicted values and no systematic deviations in the normal probability plots, indicating that the parametric ANOVA analyses were appropriate for the transformed data.

Development of light preference curve

A light preference curve ($g(L)$) was generated from the light experiments based on the probability of finding an individual mysid at a particular light intensity relative to complete darkness (see Results section for function derivation). First, we calculated the ratio between the mean proportion of mysids observed in region L and the mean proportion of mysids observed in region D for each treatment (i.e., odds of finding a mysid in region L relative to region D). Each region L:D ratio was then divided by a correction factor of 1.6 to account for the 1.6 times as many observations in region L as in region D under control (completely dark, 4 °C isothermal) conditions. This procedure corrects for mysid preference for the upper region of the columns independent of the light gradient as well as accounts for the larger volume of region L relative to region D. A best-fit line was selected that minimized the sums of squares of all observations when fit through each of the regions L:D treatment ratios (for derivation, see Results section, Experiment 1: light experiment and preference function). The resulting curve was then scaled between 0 and 1 by dividing each treatment ratio value by the maximum value of the curve. We call values on this curve relative probabilities, as each point on the curve represents the probability of observing a mysid at that particular light level relative to the most preferred light level (where the value of the curve = 1). To calculate the odds of finding a mysid at any one light level (i.e., light level 1) relative to any other light level (i.e., light level 2), the relative probabilities associated with each of the two light levels are simply divided by one another (i.e., value at light level 1 × (value at light level 2)$^{-1}$ = odds of finding a mysid in light level 1 relative to light level 2). Note that these relative probabilities are based on the ratio of observations in region L:region D and are different from the proportions used in the ANOVA analyses, which are based on the ratio of observations in region L:(regions L + D).

Experiments 2 and 3: DLPT and PLLT combination experiments

We performed both the DLPT and PLLT combination experiments to test the assumption of Gal et al. (2004) and Boscarino et al. (2007) that temperature and light preference are equally important and independent predictors of mysid vertical distribution. Pairing a deterring condition with a favored condition should provide the best test of independence of the two factors.

In the DLPT combination experiment, a deterring light intensity of 10^{-2} mylux was combined with a preferred 6 °C temperature in the upper portion of the column (Figs. 2*a* and 2*b*). The region L light level was set to 10^{-2} mylux because the light experiments indicated that mysids were deterred, but did not completely avoid these light levels. Our selection of 6 °C as the preferred temperature was based on results from the mysid temperature preference experiments performed by Boscarino et al. (2007). We ran two sets of control trials for the purpose of statistical comparisons. In the light control trials, we observed mysid vertical distribu-

Table 1. Light and temperature conditions, sampling depths and times, and comparisons of model predictions and observed mysid distributions.

	7 May 1996	6 May 1996	15 Aug. 1995	2 Aug. 1995
Corresponding Fig. 5 panel	*a*	*b*	*c*	*d*
Moonlight conditions (% illumination, moon altitude)	82%, 22°	0%, –16°	69%, 13°	0%, –28°
Bottom depth (m)	120	120	90	130
Time of sampling	0203	2200	2347	0100
Surface temperature (°C)	2.6	2.6	24.4	23.5
Thermocline depth (m)	NA	NA	19	11
Observed depth of peak mysid density (m)	49	36	23	14
Predicted depth of peak mysid density (m)	49	34	24	19
Difference from peak (m)	0	2	1	5
% overlap	70	67	75	79

Note: Percent illumination refers to the percent of the moon's disc that is illuminated, and moon altitude refers to the angle of the moon above the horizon at the time of sampling. The thermocline depth is defined as the depth at which temperature change per metre is greatest. Differences from peak values were calculated as the difference between model predictions and actual observations (in m) of the depth of maximum mysid density. Predicted values are derived from the vertical distribution model. Percent overlap is based on comparisons between model predictions and observed distributions and was calculated using Czekanowski's index.

tion in a 4 °C isothermal, dark:10^{-2} mylux gradient. In the temperature control experiments, we measured mysid position in a completely dark, 4 °C:6 °C gradient. Hereafter, these conditions will be referred to as the deterring light control and preferred temperature control, respectively. When a 6 °C upper column was combined with the 10^{-2} mylux upper column condition, we defined this as the DLPT combination treatment.

In the PLLT combination experiment, a limiting temperature of 12 °C was combined with a preferred 10^{-6} mylux intensity in the upper portion of the column (region L) (Fig. 2*b*). We used 12 °C as the limiting temperature because mysids are limited by, but do not completely avoid, this temperature (Boscarino et al. 2007). The region L light intensity was set to 10^{-6} mylux because these light levels were strongly preferred by mysids in the light experiments.

We ran two sets of control trials for the purpose of statistical comparisons. In the light control trials, we observed mysid vertical distribution in a 4 °C isothermal, dark:10^{-6} mylux gradient. In the temperature control experiments, we measured mysid position in a dark, 4 °C:12 °C gradient. Hereafter, these conditions will be referred to as the preferred light control and limiting temperature control, respectively. When a 12 °C upper column was combined with the 10^{-6} mylux upper column condition, we defined this as the PLLT combination treatment.

Vertical distribution model

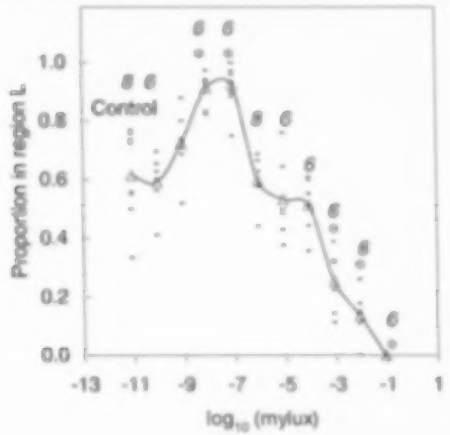
We constructed a model based on the light preference curve derived in this study, ($g(L_z)$) and the mysid temperature preference curve of Boscarino et al. (2007) ($f(T_z)$) that predicts the entire vertical distribution of mysids in a water column given ambient light and thermal conditions. Our predictions based on light and temperature preferences represent a modification of a model originally created by Gal et al. (2004) and updated by Boscarino et al. (2007) to predict mysid vertical distributions in Lake Ontario. Following these previous studies, we consider the probability of finding a mysid at given depth z (i.e., P_z) to be proportional to the product of $g(L_z)$ and $f(T_z)$ — the preference for light and temperature at depth z. The model assumes independence and equal weight of both preferences curves. Therefore, the probability of finding a mysid at any depth z, given all available depths is (1, z_{max}) equals

$$(1) \qquad P_z = \frac{g(L_z) \cdot f(T_z)}{\sum_{1}^{z_{max}} g(L_z) \cdot f(T_z)}$$

We used this model to predict mysid distributions in both our experimental columns and in the field. For comparisons in the laboratory, model predictions were compared with observed proportions of mysids in region L with two-tailed t tests ($\alpha = 0.05$) in the DLPT and PLLT combination experiments. We also compared predictions of our model with field distribution data from Lake Ontario collected on two nights with an isothermal water column in May 1996 and on two nights in which the water column was thermally stratified in August 1995. The data used in our model applications were collected at sites close but not identical to the sites published in Gal et al. (2004). All profiles were derived from data collected with a 420 kHz acoustics system (Gal et al. 2004), validated with stratified net tows, and represent sections of the acoustics data where there were no obvious fish targets. Ship lights were turned off during sampling to eliminate effects of artificial light. Comparisons were made between field and predicted distributions using the Czekanowski index of percent overlap ($[1 - [0.5\Sigma(\text{observed} - \text{predicted})]] \times 100$, Feinsinger et al. 1981) and between observed and predicted peak depth distributions of mysids.

Extinction coefficients on each night were estimated from light profiles measured the previous day with a calibrated LI-193 (Licor, Inc.) underwater spherical quantum sensor (see Gal et al. 2004). We used the relationship in Jerome et al. (1983) between average k_{PAR} and wavelength-specific extinction coefficents to calculate irradiance at depth in mylux, given the normalized spectral sensitivity curve (range of values on curve = 0 to 1) of *M. relicta* (see Gal et al. 1999). Moon phase and altitudes, sampling dates and times, and temperature conditions used in our modeling applications

Fig. 3. Proportion of all region L and region D mysid observations that were recorded in region L in the light experiments. Each replicate is represented by a dash, and mean proportions are represented by an open triangle. The solid line delineates a rolling average through the mean proportions. Control distributions are shown on the far left of the graph. Statistical comparisons were made relative to these control distributions. Asterisks indicate that the treatment was significantly different from the control proportion. Degree of significance was based on one-way analysis of variance (ANOVA) of arcsine-transformed data with Dunnett's *t* test at an experiment-wise error rate of 0.05. The number of replicates for each treatment is shown in italics above each mean proportion.



are presented (Table 1). Note that the two August and two May profiles include one night in which the moon had not yet risen above the horizon and represented starlight-only conditions and one night in which approximately three-quarters of the moon's disc was illuminated. Surface light levels were estimated with the computer program of Janiczek and DeYoung (1987) for the three-quarters moon nights and with the moonlight illuminance model of Austin et al. (1976) for the starlight-only nights.

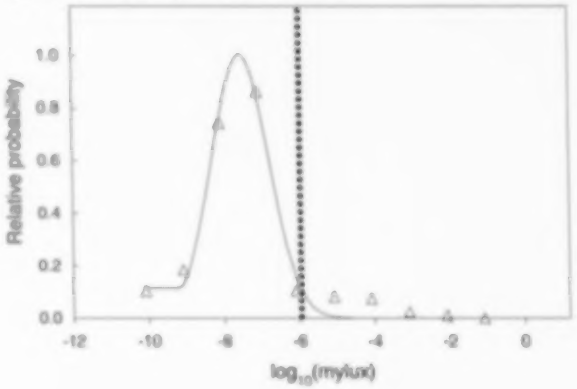
Results

Experiment 1: light experiment and preference function

Light strongly affected the proportion of mysids observed in region L relative to a completely dark region D (one-way ANOVA, $F_{[10,59]}$, $p < 0.0001$, $n = 70$, Dunnett's t test) (Fig. 3). Mysids displayed strong preferences for the 10^{-8} and 10^{-7} mylux treatments, supporting our hypothesis that mysids are attracted to a certain quantifiable range of light intensities. No significant differences in proportion were observed among dark, 10^{-10}, and 10^{-9} mylux light conditions, indicating that these low light levels are neither preferred nor avoided by mysids (Fig. 3). These low light levels may not be detectable by mysids. We observed significantly fewer mysids in light level treatments of 10^{-3} mylux or greater relative to dark columns, and no observations of mysids were recorded for light levels of 10^{-1} mylux.

A Gaussian curve based on the logarithm of light in mylux units was fitted to our experimental data for light intensities $>10^{-9}$ mylux and $\leq 10^{-1}$ mylux, which minimized the sums of squares of differences between observed and predicted region L:region D ratios (nonlinear least-squares regression, SAS statistical package version 9.1, $a = 0.76$, $L_{pref} = -7.53$, $r^2 = 0.97$) (Fig. 4). The parameter a is the standard deviation of the fitted curve ($a = 0.76$, SE = 0.06), and L_{pref} represents the preferred log light intensity of mysids as predicted by the curve ($L_{pref} = -7.53$, SE = 0.06) (Fig. 4). Therefore, the peak of the curve was found at approximately $10^{-7.53}$ mylux. The equation for this light preference function, $g(L)$, where L equals light intensity in mylux, is

Fig. 4. Mysid light preference curve from the light experiments. The Gaussian curve (solid line) is based on the logarithm of light, in units specific for mysid vision, fit to describe the probability of observing a mysid at a particular light level relative to the most preferred light level. The curve was fit through observed probabilities, which are represented by open triangles. The peak of the curve occurs at $10^{-7.53}$ mylux. The broken line represents the light level at the upper 90th percentile, or the upper light threshold for mysids (as defined by Gal et al. 2004).



$$(2)\quad g(L) = e^{-0.5\left[\frac{\log_{10}(L) - (L_{pref})}{a}\right]^2} = e^{-0.5\left[\frac{\log_{10}(L) - (-7.53)}{0.76}\right]^2}$$

Since there were no significant differences between the 10^{-9} treatment and the dark, control depth distributions in the light experiments, we set the $g(L)$ function equal to 0.11 (the function value for 10^{-9} mylux) for all light intensities $\leq 10^{-9}$ mylux . We chose this function based on the good fit to the observed, experimental data. We defined the upper threshold for mysids as the depth below which 90% of the population is observed or predicted (Gal et al. 1999, 2004). The cumulative distribution function for eq. 2 showed that this threshold corresponded to a light intensity of 2×10^{-6} mylux (Fig. 4).

Experiments 2 and 3: DLPT and PLLT combination experiments

We used our model, based on mysid light and temperature preferences (see eq. 1), to predict the percentage of mysids

Table 2. Comparison of observed versus predicted percentages of mysids in region L in the deterring light – preferred temperature (DLPT) combination and the preferred light – limiting temperature (PLLT) combination experiments.

(*a*) DLPT experiment.			
	Deterring light control	Preferred temperature control	DLPT combination
Observed	13%	96%	5%
Predicted	0.001%*	99%	0.1%
n	5	7	6
(*b*) PLLT experiment.			
	Preferred light control	Limiting temperature control	PLLT combination
Observed	91%	40%	80%
Predicted	94%	39%	81%
n	6	8	6

Note: An asterisk indicates that there was a significant difference ($\alpha = 0.05$) between predicted and observed percentages. Predicted values are derived from the vertical distribution model. The number of replicates (*n*) is shown.

found in region L of our experimental columns and compared model predictions with observed percentages in both the DLPT and PLLT combination experiments. In the DLPT combination experiments, no significant differences were observed between predicted (99%) and observed (96%) percentages of mysids in region L in the preferred temperature control trials (two-tailed *t* test; $t_{6,\ \alpha = 0.05}$, $n = 7$, $p = 0.94$); however, a larger percentage of mysids were observed in the deterring light control trials (i.e., 13%) than was predicted (<0.001%) (two-tailed *t* test; $t_{4,\ \alpha = 0.05}$, $n = 5$, $p \leq 0.0001$) (Table 2). We failed to reject the null hypothesis that the percentage of observed mysids in region L of our experimental columns (5%) equals the percentage predicted by our model (0.1%) in the DLPT combination treatment (two-tailed *t* test; $t_{5,\ \alpha = 0.05}$, $n = 6$, $p = 0.06$; Table 2).

In the PLLT combination experiments, no significant differences were observed between predicted (39%) and observed (40%) region L percentages for the limiting temperature control trials (two-tailed *t* test; $t_{7,\ \alpha = 0.05}$, $n = 8$, $p = 0.42$) and between predicted (94%) and observed (91%) region L percentages for the preferred light control trials (two-tailed *t* test; $t_{5,\ \alpha = 0.05}$, $n = 6$, $p = 0.80$; Table 2). We also failed to reject the null hypothesis that the percentage of observed mysid distributions in region L of our experimental columns (80%) equals the percentage predicted by our model (81%) in the PLLT combination trials (one-tailed *t* test; $t_{5,\ \alpha = 0.05}$, $n = 6$, $p = 0.47$; Table 2).

In summary, these results suggest that giving equal weight and independence to the two preference functions provides reasonable predictions of vertical distribution under different light–temperature combinations in the laboratory.

Comparison of model predictions with field data

Our model predicted the depth of the peak mysid density to within 2 m on the spring, starlight-only profile (6 May 1996) and predicted the peak to the exact depth when a three-quarters moon had risen above the horizon (7 May 1996). The percent overlap was also high for both of these spring nights (70% and 67% for the three-quarters moon and starlight night, respectively). Our model predicted the depth of the peak mysid density to within 1 m on the 15 August 1995 night and to within 5 m on 2 August 1995 when the water column was thermally stratified. The percent overlap was also high for both the three-quarters moon and starlight-only profiles in August (75% and 79%, Figs. 5*c* and 5*d*, respectively).

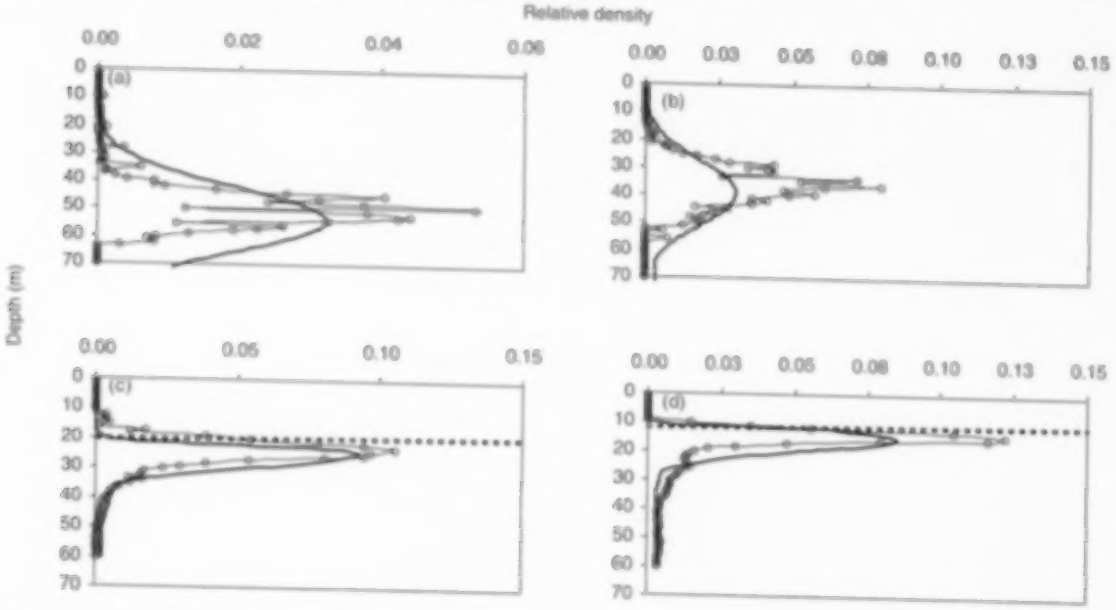
Discussion

The ability to predict a migrating population's vertical distribution from readily measurable parameters such as light and temperature has important implications for aquatic food web models. Researchers have relied on temperature- and light-based optimization models to predict the depth at which migrating organisms would maximize either the foraging gain:predation risk ratios (Clark and Levy 1988; Scheuerell and Schindler 2003), or overall consumption and growth rates (Levy 1990*a*, 1990*b*). However, migrating populations occupy a range of depths (not just one "optimal" depth), and many of these optimization models do not predict entire distributions. Therefore, they are not able to account for the feeding and behavioral interactions that often take place at the edges of a population's vertical distribution (Stuntz and Magnuson 1976). Other models that build on evolutionary game theory (Iwasa 1982; Gabriel and Thomas 1988), ideal free distribution theory (Larsson 1997; Lampert et al. 2003; Kessler and Lampert 2004), and individual-based, neural network genetic algorithms (i.e., Huse and Giske 1998; Huse et al. 1999) do predict entire distributions of migrating organisms. However, these models often rely on idealistic assumptions (e.g., a lack of predators for migrating *Daphnia* to display an ideal free distribution with costs; Kessler and Lampert 2004; Lampert 2005), on complex algorithms that require many parameters with intensive data requirements (e.g., Huse and Giske 1998), or require knowledge of behavioral variability among individuals in the population (e.g., Giske et al. 2003). Therefore, these models are not easily applied to field distributions or to those systems in which these data are not available.

In this study, we show that a model based on two readily measured environmental parameters, light and temperature, provides a reasonable prediction of mysids under both thermally stratified and isothermal, as well as new moon and moonlit, conditions. The functions used in our model are based on direct observations of mysid responses to controlled light and temperature conditions in the laboratory. This approach represents an important advance over previous modeling efforts that employed a light preference curve derived from field distributions (see Gal et al. 2004; Boscarino et al. 2007) — a preference curve that may not have been independent of the distribution used to test the model and did not take into account other potential influencing factors, such as predator and prey densities or distributions. We therefore believe that the light preference curve derived in this study more accurately describes the specific influence of light on habitat selection of mysids.

The experimental procedure we used to determine light preferences of mysids is similar to that used by Swift and

Fig. 5. The observed and predicted vertical distribution of mysids on Lake Ontario. Acoustic profiles were taken at (*a*) 0203 on 7 May 1996 when a three-quarters moon had risen above the horizon, (*b*) 2200 on 6 May 1996 before moonrise, (*c*) 2347 on 15 August 1995 when a three-quarters moon had risen above the horizon, and (*d*) 0100 on 2 August 1995 after moonset. The distributions are given as relative densities; therefore, the total density for a given profile equals one. Model predictions (bold line) were made through application of eqs. 1 and 2 to independently collected acoustic data. Observed distributions are delineated by the solid line with an open circle. The depth of the thermocline on both summer nights is represented by a horizontal, broken line.



Forward (1980, 1982) on the midge larvae *Chaoborus punctipennis*, and by Forward (1974) and Forward et al. (1984) on the crab larvae *Rhithropanopeus hariisii* — responses to different light intensities were quantified based on relative proportions of organisms in an illuminated section of an experimental column versus a section furthest from the lighted region. Unlike these previous studies that have focused specifically on the photoresponse mechanisms of migrating zooplankton (see also Forward 1988 and Ringelberg 1999 for good reviews), our results are used to derive a light preference curve capable of predicting field distributions. However, it is important to note that while our light preference curve is capable of predicting field distributions, the light experiments were not designed to test actual phototactic responses of mysids. Forward (1988) provides a detailed review of the complications associated with quantifying the photoresponses of organisms to a unidirectional source of light. For example, Stearns and Forward (1984*a*) demonstrate that the marine copepod *Acartia tonsa* is positively phototactic (i.e., moves towards light) when given a directional light source such as a slide projector, but shows a different response when a natural, underwater angular distribution of light is simulated (Stearns and Forward 1984*b*). In our experiment, we project a collimated beam of light horizontally onto our experimental columns and therefore cannot state that mysids are responding phototactically (i.e., either moving towards or away from a source of light). Our experiments only reveal the preference for different light levels relative to complete darkness; therefore, we believe that our experimental results are more indicative of mysid light preference and avoidance rather than providing a direct measure of phototaxis.

We believe that the light and temperature conditions we implemented in our experimental columns are comparable with those experienced by mysids in the field. Light level treatments spanned a range of light intensities available to mysids from dusk until dawn. The highest light treatment (10^{-1} mylux, or 1 lux) is comparable with dusk and dawn conditions at the surface of Lake Ontario (B. Boscarino, personal observation) and therefore represents the highest light level a mysid would encounter during vertical ascent. Experimental treatments were also extended to complete darkness to provide control conditions for statistical comparisons, as well as to help determine a lower threshold light level at which mysids do not demonstrate a behavioral response. Additionally, the light meter used to record the intensity of light available to mysids was fitted with a filter that closely matched the relative sensitivity of the mysid eye to different wavelengths of light.

The preferences and deterrences we observed for mysids in the laboratory are very similar to those reported for mysids in the field. The peak of our preference curve occurred at $10^{-7.53}$ mylux, which is within a factor of five of the peak of the preference curve derived by Gal et al. (2004). Our laboratory-derived upper light threshold of 2×10^{-6} mylux is identical to the light levels associated with the leading edge of *M. relicta* migratory layers in Lake Ontario (2×10^{-6} mylux) and closely coincides with that of *M. mixta* in the Baltic Sea (10^{-4} lux, or $\sim 10^{-6}$ mylux — see Gal et al. 2004 and Rudstam et al. 1989, respectively). Other studies

report slightly higher threshold light values (0.02–0.05 lux, or 10^{-4} mylux in Green Lake, Wisconsin; Teraguchi et al. 1975). However, in situ measurements of the water chemistry and (or) spectroradiometric data at each depth interval at the time of sampling are rare (but see Gal et al. 1999), and estimates of both the spectral quality and overall intensity of light at depth are typically off by an order of magnitude or more if these factors are not accounted for, as they have been in this study (i.e., Widder and Frank 2001).

Light is not the only factor influencing distribution, and this study demonstrates the importance of the interaction between temperature and light in influencing depth selection of mysids. Previous models of mysid distribution (e.g., Gal et al. 2004; Boscarino et al. 2007) assumed that temperature and light functions were independent and had equal weight. Although the influences of light and temperature on mysid distribution may indeed be independent as the response may be related to different processes (i.e., predator avoidance and growth maximization), there is no similar a priori reason why giving equal weight to the two functions is more likely than another weighing factor. However, our laboratory results in combined light and temperature gradients suggest that these assumptions are at least reasonable. The distributions of mysids in the combined gradients were in most cases not significantly different from model predictions, with the one exception of the deterring light control trials, when a larger proportion of mysids were found in region L than was predicted. It is possible that our light function may underestimate the number of mysids in these light levels.

Given that our model was constructed based on the preferences of mysids 12 mm or larger, our preference curve may not be applicable to mysids in other size classes. Juvenile mysids, for example, may have different light and temperature preferences than adults. Bowers (1988) demonstrated that the majority of the mysid population between 0 and 50 m in Lake Superior was less than 7 mm, whereas adults greater than 13 mm were only caught at depths greater than 50 m. Similar vertical separation between adult and juvenile mysids have been reported for *M. mixta* in the Baltic Sea (Salemaa et al. 1986; Rudstam et al. 1989) and for *M. relicta* in Lake Michigan (Grossnickle and Morgan 1979), suggesting that smaller mysids may have higher light and temperature tolerances than larger mysids. For example, Morgan and Threlkeld (1982) demonstrated that only juvenile mysids were capable of undergoing horizontal migrations into warmer, nearshore waters in the summer, suggesting different thermal tolerances of adults and juveniles. Not accounting for juvenile mysids in our vertical distribution model may lead to an underestimation of the upper limit of the mysid distribution. This explanation may account for the underestimation of the upper extent of the mysid migratory layer on 15 August 1995. However, we were able to accurately predict both the range and peak of mysid vertical distribution in Lake Ontario for most of the sampling nights we analyzed, suggesting that either size differences were not playing a large role in structuring the overall distribution, or our acoustic sampling procedure did not accurately detect mysids <12 mm. We are currently investigating the relative contribution of smaller mysids to overall backscattering at the upper edges of mysid migratory layers as well as deriving temperature and light preferences for juvenile mysids. These investigations should help clarify ambiguities regarding potential differences in thermal and light preferences as well as depth selection differences of mysid age classes.

The low light preferences of mysids obtained in this study may place them tens of metres below a denser epilimnetic zooplankton layer (Reynolds and DeGraeve 1972; Gal et al. 2006). This degree of separation between mysids and zooplankton will vary by moon phase. For example, Rybock (1978) found mysids well below the zooplankton layer in Lake Tahoe on full moon nights, but closer to the zooplankton layer on new moon nights. Beeton and Bowers (1982) hypothesized that full moon conditions should therefore inhibit a mysid's ability to feed on zooplankton and limit their impact on the pelagic food web. In addition to decreased spatial overlap with their prey, lower light levels may decrease capture success. Ramcharan and Sprules (1986) reported significantly higher mysid feeding coefficients at light levels between 1 and 10^{-2} mylux (assuming a conversion factor of 1 mylux = 0.51 $W \cdot m^{-2}$) compared with feeding rates under dark conditions. These results suggest that a mysid's choice of low light habitat inhibits its ability to locate and successfully capture zooplankton. These inhibitory effects of light are likely to be most pronounced during isothermal conditions, when light is the primary abiotic factor influencing distribution (Johannsson et al. 2003; this study). Given that Lake Ontario is isothermal for much of the year (typically from early to mid-November to early June; Schertzer 2003), seasonal changes (i.e., moon phase, algal productivity), as well as long-term shifts in light regime (i.e., oligotrophication or eutrophication) are likely to play a central role in determining the degree of overlap between mysids, their prey, and predators and in structuring Lake Ontario's pelagic food web dynamics in general.

Given the apparent sacrifice in terms of prey consumption associated with choosing low light habitats, there must be a strong evolutionary pressure for selecting these types of environment. The most likely reason is that low light preferences of mysids evolved as an adaptation to avoid predation by visual predators like fish. Alewives are a main predator of mysids throughout the Great Lakes and typically remain in epilimnetic waters from late spring to early fall in these systems (Olson et al. 1988; O'Gorman et al. 2000). Although alewife can feed on mysids in the dark (Janssen et al. 1995), feeding rates decline relative to lighted conditions. Batty et al. (1990) offered a mixture of zooplankton to the Atlantic herring, *Clupea harengus*, which ceased particulate feeding at 0.001 lux (i.e., approximately 10^{-5} mylux) — a light level that is almost 200 times greater than those most preferred by mysids and three times the upper light threshold derived for mysids in this study. However, when fed *Artemia* nauplii, herring ceased particulate feeding at 0.01 lux, or $\sim 10^{-4}$ mylux, indicating that feeding thresholds for fish can vary depending on the prey item that is used (Batty et al. 1990). Given that mysids are a much larger prey item than the zooplankton used by Batty et al. (1990), mysids may be easier to see under low light conditions, and therefore the light threshold for fish visual feeding may be slightly lower when feeding on mysids. It should also be noted that while alewives have been one of the most abundant planktivores in the Great Lake system for the past few

decades, they are a relatively recent invader (Miller 1957), and mysids have coexisted with coldwater predators, such as *Coregonus* spp., for a much longer period of time. This coexistence is therefore likely to have had an even greater influence on shaping the habitat preferences and antipredatory behaviors of mysids than alewife. Little is known about coregonid feeding at low light levels, but Janssen (1980) has shown that lake herring (or cisco), *Coregonus artedii*, are capable of feeding on *Daphnia* in complete darkness, although they can only do so in a nonselective manner. However, capturing larger, strong-swimming invertebrates such as *M. relicta* requires selective particulate feeding, which is thought to be a primarily vision-oriented behavior in coregonids (Janssen 1978). The light threshold required to switch from a nonselective to a particulate feeding mechanism in both coregonids and alewife is still unknown. We cannot, therefore, conclude the precise light levels at which mysids can safely avoid visual predation from coregonids, and future investigations would be necessary to provide estimates of coregonid feeding rates in low light conditions.

This study extends previous efforts by Gal et al. (2004) and Boscarino et al. (2007) to describe and model the responses of mysids to light and temperature gradients. These previous modeling efforts have relied on extrapolation from field distributions to predict the effect of light on vertical distribution and did not test for preference under controlled conditions. We argue that the derivation of a function based on laboratory observations of mysid depth selection behavior given different quantifiable light gradients is a more accurate method of determining light preference than field extrapolations, which may be sensitive to errors in acoustic estimates and (or) light estimation. Successful application of our laboratory-derived model to predict independently collected field distributions further supports the light preferences we observed for mysids in the laboratory.

Given recent increases in water clarity associated with oligotrophication and the dreissenid mussel invasions of many of the ecosystems inhabited by mysids (e.g., Mills et al. 2003), as well as the potential impacts of global climate change on the thermal structure of deepwater lakes of North America (Magnuson et al. 2000; Schindler et al. 2005), it is important that we begin to understand how such long-term shifts in environmental conditions may be impacting the distributions and behaviors of the biotic community inhabiting these systems, including mysids. Similar models to those derived in this study have been used to forecast impacts of climate change on the vertical and horizontal distributions of migrating organisms (e.g., DeStasio et al. 1996; McDonald et al. 1996; Schindler et al. 2005). We believe that our model can also be used to predict how global-warming-mediated thermal changes and shifts in light regime may impact food web dynamics via alteration of habitat use by *Mysis relicta*. These predictions may be made in any deepwater ecosystem that mysids inhabit where the main mysid predators are primarily visual-feeding fish.

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A biophysical model of Lake Erie walleye (*Sander vitreus*) explains interannual variations in recruitment

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Abstract: We used a three-dimensional coupled hydrodynamic–ecological model to investigate how lake currents can affect walleye (*Sander vitreus*) recruitment in western Lake Erie. Four years were selected based on a fall recruitment index: two high recruitment years (i.e., 1996 and 1999) and two low recruitment years (i.e., 1995 and 1998). During the low recruitment years, the model predicted that (*i*) walleye spawning grounds experienced destructive bottom currents capable of dislodging eggs from suitable habitats (reefs) to unsuitable habitats (i.e., muddy bottom), and (*ii*) the majority of newly hatched larvae were transported away from the known suitable nursery grounds at the start of their first feeding. Conversely, during two high recruitment years, predicted bottom currents at the spawning grounds were relatively weak, and the predicted movement of newly hatched larvae was toward suitable nursery grounds. Thus, low disturbance-based egg mortality and a temporal and spatial match between walleye first feeding larvae and their food resources were predicted for the two high recruitment years, and high egg mortality plus a mismatch of larvae with their food resources was predicted for the two low recruitment years. In general, mild westerly or southwesterly winds during the spawning–nursery period should favour walleye recruitment in the lake.

Résumé : Nous utilisons un modèle tridimensionnel hydrodynamique et écologique couplé pour étudier comment les courants lacustres peuvent affecter le recrutement du doré (*Sander vitreus*) dans la région occidentale du lac Érié. Nous avons retenu quatre années d'après un coefficient de recrutement automnal, deux années de fort recrutement (1996 et 1999) et deux années de faible recrutement (1995 et 1998). Les années de recrutement faible, le modèle prédit que (*i*) les frayères de dorés ont connu des courants de fond destructeurs capables de déloger les œufs des habitats favorables (récifs) et de les reléguer dans des habitats inadéquats (fonds boueux) et (*ii*) la majorité des larves nouveau-nées ont été délogées des nourriceries favorables connues au début de leur première alimentation. À l'inverse, les deux années de recrutement important, les courants de fond prédits sur les frayères étaient relativement faibles et les déplacements prédits des larves nouvellement écloses se faisaient vers les nourriceries favorables. Ainsi, le modèle prédit une faible mortalité des œufs due à la perturbation et une coïncidence temporelle et spatiale entre les larves de dorés et leurs ressources alimentaires lors de leur première alimentation pour les deux années de fort recrutement, alors qu'il prédit une forte mortalité des œufs et une séparation des larves de leurs ressources alimentaires lors des deux années de recrutement faible. En général, des vents doux de l'ouest ou du sud-ouest durant les périodes de fraye et de nourricerie devraient favoriser le recrutement des dorés dans le lac.

[Traduit par la Rédaction]

Introduction

Walleye (*Sander vitreus*) populations in Lake Erie have supported substantial fisheries for nearly 200 years, and for much of this period, these populations have exhibited large fluctuations in abundance (Schneider and Leach 1977; Lake Erie Committee – Walleye Task Group (LEC–WTG) 2006). Variation in annual recruitment is the primary factor responsible for these fluctuations (Busch et al. 1975; Shuter and Koonce 1977), and several processes have been suggested as possible mechanisms to explain this variation. Two processes that have received recent attention (Roseman et al. 1999, 2005) are (*i*) variable survival of eggs from fertilization to hatch because of disturbance of spawning habitat and (*ii*) variable survival of hatched larvae because of transport of planktonic larvae to suitable or unsuitable habitats.

For many fish species, the survival of eggs and larvae can be influenced by many environmental factors, including pre-

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dation risk and availability of suitable habitat (Bailey and Houde 1989; Bradbury and Snelgrove 2001). Among-year variability in these environmental conditions gives rise to the weak relationship between spawner stock size and subsequent recruitment commonly observed in fish populations (Myers and Barrowman 1996; Myers et al. 1997), and Lake Erie walleye provide a typical example of this phenomenon. Consequently, some environmental factors, such as water temperature, are sometimes explicitly taken into consideration in analyses of stock–recruitment relationships (Hilborn and Walters 1992). Shuter and Koonce (1977) and Madenjian et al. (1996) found a much clearer stock–recruitment relationship for Lake Erie walleye after correcting for variation in spring water warming rate. Shuter et al. (2002) showed that this masking association between recruitment variation and variation in warming rate was consistently evident over the period from 1947 though 2000, despite large systematic changes in other environmental factors, such as water levels and nutrient concentrations. Busch et al. (1975) and Roseman et al. (1999) also identified spring warming rate as an important factor influencing walleye recruitment; higher water warming rates were associated with higher recruitment.

In western Lake Erie, walleye spawn on midlake reefs in the western basin as well as in tributaries such as the Maumee, Sandusky, and Detroit rivers. Since walleye typically spawn at depths less than 5 m, many discrete reef spawning areas of various sizes are available to them; reef areas above the 5 m contour range in size from 0.01 to 1.5 km^2 (Busch et al. 1975; Roseman et al. 1996). Spawning typically begins shortly after ice-out and peaks around the middle of April (Baker and Manz 1971; Roseman et al. 1996). Walleye broadcast their eggs over hard substrates and provide no direct parental protection. Eggs typically hatch in 20 to 30 days depending on water temperature (Colby et al. 1979; Roseman et al. 1996). Small interstitial spaces in the substrates (such as reefs) provide benthic eggs some protection from predation and displacement, but eggs remain vulnerable to physical processes such as changes in water temperature, wind-induced wave and current action, and sedimentation. These processes can influence the duration of incubation, egg abundance, and survival (Roseman et al. 1996).

Upon hatching, western Lake Erie walleye larvae rely on surface water currents to reach their nursery grounds (Nepszy et al. 1991). The yolk sac is usually absorbed within 5 days, when the larvae are about 9.5 mm total length (Roseman 1997). The duration of the pelagic larval stage is temperature-dependent but typically lasts around 3 weeks, after which the young fish become demersal at a total length of about 30 mm (McElman and Balon 1979). Because of their small size and limited mobility, larval walleye can be influenced by physical processes, such as water temperature and wind-generated currents. These processes can influence walleye larval development, distribution, growth, and survival, especially during the first several days after hatching when their weak swimming ability prevents them from actively searching for suitable habitat (i.e., nursery grounds).

Walleye egg and larval surveys carried out by Roseman et al. (1999, 2001) revealed that strong water currents on walleye spawning grounds in western Lake Erie generated by strong north–northeast winds relocated walleye eggs from suitable hatching habitat (i.e., coarse substrate on the reef surface) to unsuitable muddy bottom areas adjacent to the reefs. This dislodgement caused high egg losses and low recruitment. This finding echoed that of Busch et al.'s (1975) that low egg survival was associated with strong winds observed at spawning sites during incubation periods.

Besides affecting egg survival through catastrophic disturbance of spawning habitat, water currents also control the transfer of larvae to suitable nursery grounds from incubation grounds. This transfer process is believed to be an important determinant of larval walleye survival and thus recruitment (Roseman et al. 1999, 2001, 2005). Investigations conducted by Roseman et al. (2005) during the 1990s found that densities of zooplankton, ichthyoplankton, and pelagic walleye larvae were consistently higher at nearshore sampling sites along the Maumee River plume. Given the shallow depths (less than 5 m) of the nearshore water and the fact that spring temperatures of nearshore water and Maumee River are similar, it is reasonable to assume that rive and lake water mix easily where they come in contact, thus permitting lake currents to transport larvae directly into these more productive inshore waters. Roseman et al.'s (2005) observations suggested that this region is of sufficient quality to support high numbers of young walleye, on a per unit area basis, and of sufficient magnitude to provide high absolute numbers of young walleye to the adult population (Dahlgren et al. 2006). These investigators concluded that this region is the primary nursery habitat supporting the western basin walleye population. Given this premise, it is clearly critical for walleye larvae to reach this region early in life to maximize their survival. For walleye originating from western Lake Erie reefs, water current may play an important role in shaping walleye recruitment by serving as the connective mechanism between egg incubation and hatching sites on the reefs and primary south shore nursery habitats. The potential role of currents in shaping recruitment success is accentuated by the fact that transport of walleye to the north and east of the spawning reefs would carry them to clearly unsuitable habitat where food resources are low, predator abundance is high, and water temperatures are generally lower, thus slowing growth and increasing the period over which larvae are vulnerable to predation.

Unfortunately, there are few historical field observations of water currents in walleye spawning and nursery grounds in western Lake Erie that can be used to directly test these possibilities. An alternative to direct observation is to use a physical model to assess the possible impacts of currents on walleye recruitment. In this study, we report our initial use of such a model to test two hypotheses regarding the causes leading to high and low recruitment years for western Lake Erie walleye. We used the coupled hydrodynamic – water quality models ELCOM–CAEDYM (Estuary and Lake and Coastal Ocean Model – Computational Aquatic Ecosystem Dynamics Model) (Center for Water Resources, University of Western Australia: www.cwr.uwa.edu.au/services/models.php?mdid=6) to determine if (*i*) poor recruitment years are associated with unusually high currents during the egg incubation period; and (*ii*) poor recruitment years are associated with current patterns that transport postswim larvae to unsuitable habitat, while good recruitment years are associated

Table 1. Observed spawning period (i.e., start to end of egg presence on the reefs), peak hatching for walleye (*Sander vitreus*) in main spawning ground (south reef complex), spring warming rates from 1 April to 15 May, and recruitment index (i.e., number of walleye per hour trawling from interagency fall survey) of western Lake Erie for four simulated years.

	Low recruitment years		High recruitment years	
	1995	1998	1996	1999
Spawning period[a]	1 April – 15 May	23 March – 4 May	8 April – 13 May	1 April – 6 May
Peak hatching[a]	3 May	20 April	20 May	26 May
Warming rate[a] (°C)	0.16	0.24	0.22	0.18
Recruitment index[b]	2.67	13.69	93.13	58.18

[a]From Roseman 2000.
[b]From LEC–WTG 2006.

with current patterns that transport larvae to suitable nursery habitat. ELCOM is a three-dimensional numerical modelling tool that applies hydrodynamic and thermodynamic principles to simulate the temporal and spatial behaviour of stratified water bodies when subject to defined patterns of environmental variation (Hodges et al. 2000). ELCOM has been used to model water temperature and water current patterns in numerous water bodies (Lake Kinneret: Laval et al. 2003; Lake Kinneret and Lake Ogawara: Dallimore et al. 2003; Great Slave Lake: Leon et al. 2007), including Lake Erie. Leon et al. (2005) recently completed an evaluation of the ability of ELCOM to simulate Lake Erie hydrodynamics, given observed daily variation in weather. Their study showed that ELCOM successfully predicted observed temporal and spatial patterns for both water temperature and water current for 2 years: 1994 and 2001. The positive results from their study provided the scientific justification for using ELCOM to explore the potential impacts of hydrodynamic forces on walleye recruitment in Lake Erie.

Materials and methods

Selection of recruitment years for the simulations

Four recent years were selected to represent 2 years of high walleye recruitment (1996 and 1999) and 2 years of low walleye recruitment (1995 and 1998). We selected these years by comparing age-0 walleye abundance indices from interagency trawling surveys conducted in western Lake Erie during the fall (LEC–WTG 2006). These 4 years provide considerable contrast in walleye recruitment (i.e., on average, recruitment in the high years is more than 20 times the recruitment in the low years; Table 1) and also have the desirable attribute that consistent recruitment patterns were observed for these 4 years from both Ontario and Ohio trawling surveys, which suggests basin-level (rather than local) differences in walleye recruitment. In addition, data on walleye spawning time, spawning locations, and peak hatching time for these 4 years were available from Roseman et al. (1996, 2005).

The ELCOM–CAEDYM modelling framework

In the coupled ELCOM–CAEDYM model, ELCOM is the hydrodynamic driver. Its spatially explicit, three-dimensional simulations of current and temperature patterns are based on processes that include baroclinic and barotropic responses, rotational effects, tidal forcing, wind stresses, surface thermal forcing through solar input, water inflows and outflows, and transport of solute content, heat, and other passive scalars. It uses observed meteorological time series data (i.e., air temperature, relative humidity, cloud cover, atmospheric pressure, long- and short-wave radiation, wind speed, wind direction, and rainfall) plus the known bathymetry of the study lake to generate spatially explicit predictions of temporal changes in water temperature and water current. These predictions establish the physical context used by the CAEDYM water quality model to generate spatially explicit predictions of the temporal behaviour of various chemical and biological lake characteristics.

CAEDYM has a modular structure permitting users to include one or several submodels that simulate such processes as nutrient cycling, growth of phytoplankton and zooplankton, and sediment resuspension. The choice of processes to simulate depends on the interests of the user. ELCOM and CAEDYM together are then able to generate spatially explicit simulations of temporal variation in such lake characteristics as oxygen concentration, nutrient (e.g., N, P) concentrations, algal production, and (or) zooplankton production.

In our application, we use a very simple version of CAEDYM to model the travel paths of walleye larvae floating near the lake surface (Hipsey et al. 2004). This is done by keeping track of the larval concentration in each spatial unit (cell) of the model and assuming larval movement patterns will be driven by the between-cell exchanges of water predicted by ELCOM. Detailed descriptions of the physical principles and algorithms embodied in ELCOM are available in the ELCOM science and user manuals at www.cwr.uwa.edu.au/services/models/elcom/elcom_docs.php. Similar information for CAEDYM is available at www.cwr.uwa.edu.au/services/models/caedym/caedym_docs.php

Input data and model setup

High temporal resolution (hourly or 3-hourly) data on climate variables, including wind speed, wind direction, air temperature, solar radiation, relative humidity, rainfall, and cloud cover, are necessary inputs to ELCOM–CAEDYM. Because of Lake Erie's large size, climate conditions in one area can be different from other areas at the same time. To incorporate such spatial variability of inputs, we divided the lake into four areas: West Basin, western Central Basin, eastern Central Basin, and East Basin. For each area, one weather station was selected as a source of meteorological data: West Basin – Toledo, Ohio; western Central Basin –

Cleveland, Ohio; eastern Central Basis – Erie, Pennsylvania; and East Basin – Buffalo, New York. We obtained data (hourly or 3-hourly observed values except for solar radiation) from each station from March 1 to June 30 for each of 1995, 1996, 1998, and 1999 from the National Oceanic and Atmospheric Administration (NOAA) – National Climate Data Center 2006). This time period covers the typical spawning, egg incubation, and pelagic larval stages of walleye in western Lake Erie.

Because data on solar radiation were not available for those four stations, we addressed this deficiency by calculating hourly theoretical values based on station locations, elevations, the sun's position in the sky, a Linke turbidity factor (Kasten 1996), and observed cloud cover. The position of the sun in the sky varies both hourly and seasonally and is also location-dependent (latitude and longitude). The sun's position for each hour of each day was calculated using an algorithm described in Szokolay (1996). Extraterrestrial irradiance (i.e., solar radiation incident outside the earth's atmosphere) was calculated based on the solar constant (i.e., 1367 $W{\cdot}m^{-2}$) (Page 1986) and the earth–sun distance that varies slightly across a year (Paltridge and Platt 1976; Duffie and Beckman 1991). Beam irradiance normal to the solar beam was attenuated by the cloudless atmosphere and calculated based on extraterrestrial radiation, Linke turbidity factor, relative optical air mass (Kasten and Young 1989), and Rayleigh optical thickness. Diffuse irradiance was calculated based on extraterrestrial irradiance, the diffuse transmission function dependent on the Linke turbidity factor, and the diffuse solar altitude function dependent on the solar altitude (Scharmer and Greif 2000). The global irradiance under clear sky was the sum of beam irradiance and diffuse irradiance. The global irradiance under real sky (i.e., observed cloudy sky) was derived from the global irradiance under clear sky calculated above and observed hourly cloud cover values (Kasten and Czeplak 1980). For the past 20 years, the monthly average values for the Linke turbidity factor for Lake Erie were obtained from the SoDa web site (www.soda-is.com/eng/index.html), and daily values were interpolated from those monthly means.

Daily average flow rates from the three main tributaries of the lake were used as sources of the inflow (Detroit River and Maumee River) or outflow (Niagara River) inputs for the simulation. Flow rates for the Maumee River and the Niagara River were obtained from the USGS National Water Information System (waterdata.usgs.gov/nwis/sw). The daily flow rate for the Detroit River was only available for 1976–1977. Monthly means for the Detroit River flow rates from 1900 to 1999 were available from the NOAA Great Lake Environmental Research Laboratory (www.glerl.noaa.gov/data/arc/hydro/mnth-hydro.html). Thus, the daily flow rates for the Detroit River for modeling years (i.e., 1995, 1996, 1998, and 1999) were derived by adjusting daily flow rates for 1976–1977 using the differences of monthly means between each modeling year and year 1976–1977. A three-dimensional bathymetric map of Lake Erie distributed by the National Geophysical Data Center (www.ngdc.noaa.gov/mgg/greatlakes) was used to interpolate the lake bathymetry into a 2 km by 2 km raster format that was also used for model input. The simulation was run at a time step of 20 min, and the output variables (i.e., water temperatures and currents at the lake bottom and surface, plus larvae concentrations) were reported at 3 h intervals.

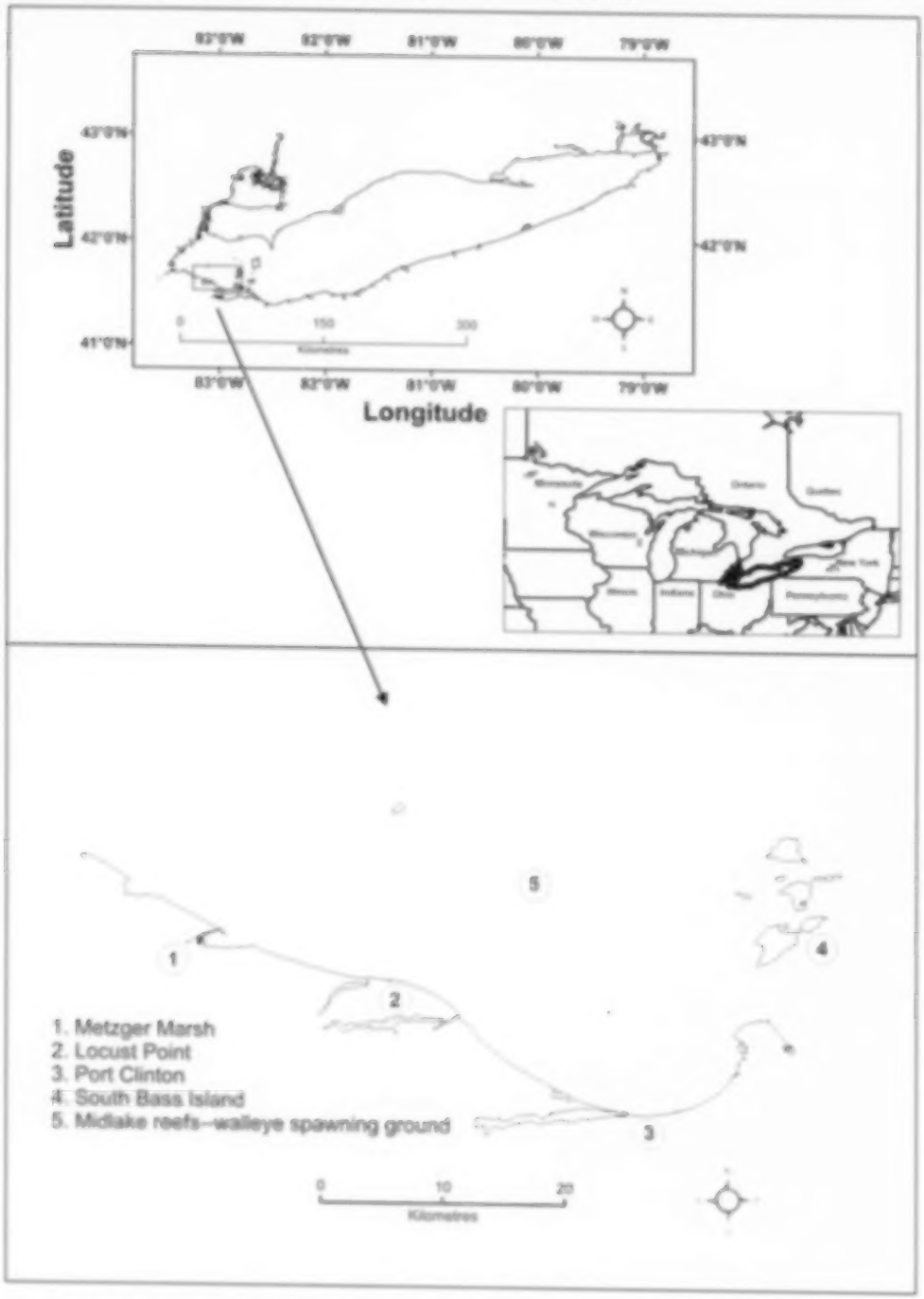
The spawning and hatch areas for walleye in western Lake Erie were defined from the Roseman et al. (1996, 2001) field surveys. Suitable nursery ground in the western basin of Lake Erie was also derived from the same surveys (Roseman et al. 2005; Fig. 1).

During the simulation, we monitored the simulated water currents at the spawning grounds through the incubation and hatching periods. We assumed that the mean incubation time would be 30 days from the initial date of spawning (Busch et al. 1975; Roseman et al. 1999). Roseman et al. (2005) observed that the temporal pattern of walleye hatching exhibited a single, distinct peak in each of our study years. For simplicity, we assumed that the cohort of individuals hatching on this peak date in each year would be the primary determinant of walleye recruitment for the year and thus focused our study on the transportation pattern of this "peak hatched cohort". We simulated this travel path for each year by releasing particles representing walleye larvae at the spawning ground labeled in Fig. 1 at the observed date of the peak hatching (Roseman et al. 2005) for each year. Houde (1969) found that larval walleye less than 7.9 mm (around first month) in length (i.e., age from hatch $< \sim 30$ days) have barely detectable swimming abilities, and it is known that walleye larvae this young are positively phototactic (Ryder 1977) and are typically found in surface waters (Roseman et al. 2005). Therefore, we assumed that walleye larvae during the period of interest (i.e., first 5 days after peak hatching date) could be represented in the model as positively buoyant, passive particles. We monitored variations in the concentration of larvae-like particles dispersed to the suitable nursery habitats during the 5-day period after peak hatching because this is the time period over which the majority of larvae would have started their first feeding (Roseman 1997). We compared the implied patterns of dispersion from the spawning ground with nursery habitats among years and related them to age-0 recruitment indices.

Results

Among the four simulated years, walleye initial spawning dates varied from March 23 to April 8 (Table 1). During the typical observed walleye incubation period in Lake Erie (i.e., April 1 to May 15; Roseman 2000), daily average wind speed and simulated water currents at the reefs were highly variable both among years and within each year (Fig. 2), as was wind direction (Fig. 3). The predominant wind direction during this period was from the northeast, except for 1996, which was dominated by southwest winds (Fig. 4). Northeast winds tended to be associated with stronger bottom currents at the spawning sites than other wind directions (Figs. 2, 3). For 1998, our model predicted that strong northeast winds around April 10–11 would have generated strong bottom currents ($> \sim 0.19$ $m{\cdot}s^{-1}$) at this time (Fig. 2), coincident with the observed high loss of walleye eggs in this year (Roseman et al. 2001). The model predicted a similar magnitude of bottom current for the spawning season in 1995, which is the other low recruitment year (Fig. 2). In the high recruitment years (1996 and 1999),

Fig. 1. Great Lakes region (inset map in top panel), with western Lake Erie main walleye (*Sander vitreus*) nursery grounds (indicated by the rectangle) and spawning grounds (lower map) shown (modified from Roseman et al. 2005).



wind conditions during walleye spawning did not result in observed significant egg losses like in 1998, and for these years, simulated bottom current was $< \sim 0.12$ m·s^{-1} (Figs. 2, 3). These results suggest that the local turbulence (i.e., turbulence stemming from wave surge as well as currents) associated with a simulated bottom current speed $> \sim 0.19$ m·s^{-1} is sufficient to cause substantial mortality of walleye, while turbulence associated with a simulated bottom current speed

Fig. 2. Wind speeds (broken line) and predicted bottom water currents (solid line) at the main walleye spawning ground (south reef complex) during the presumed egg incubation period for (*a*) 1995, (*b*) 1996, (*c*) 1998, and (*d*) 1999. The star indicates the time during 1998 when catastrophic egg losses from the reef were observed (Roseman et al. 2001).

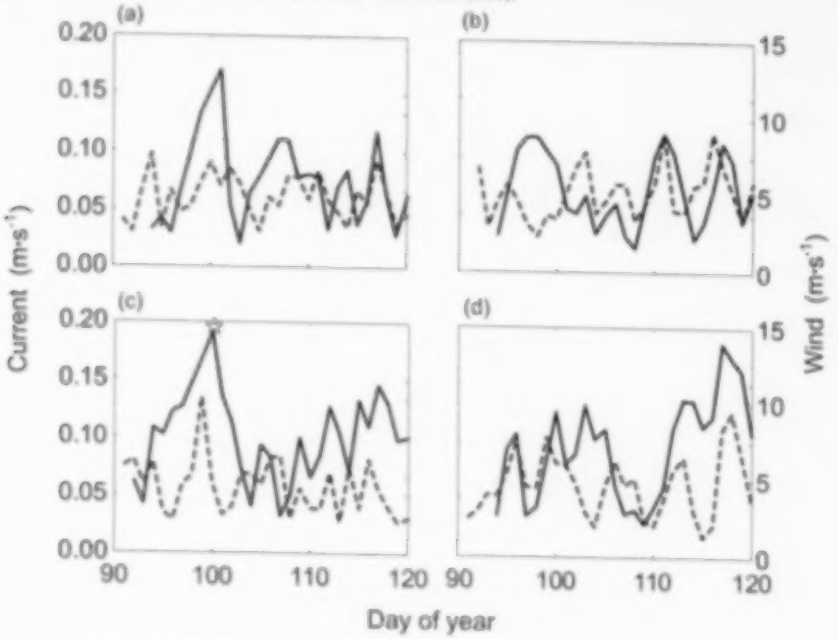
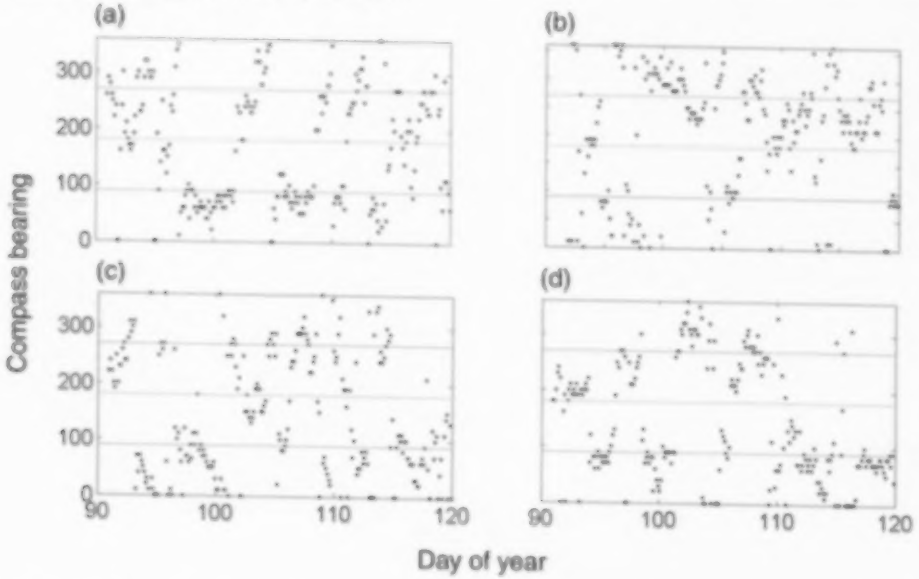


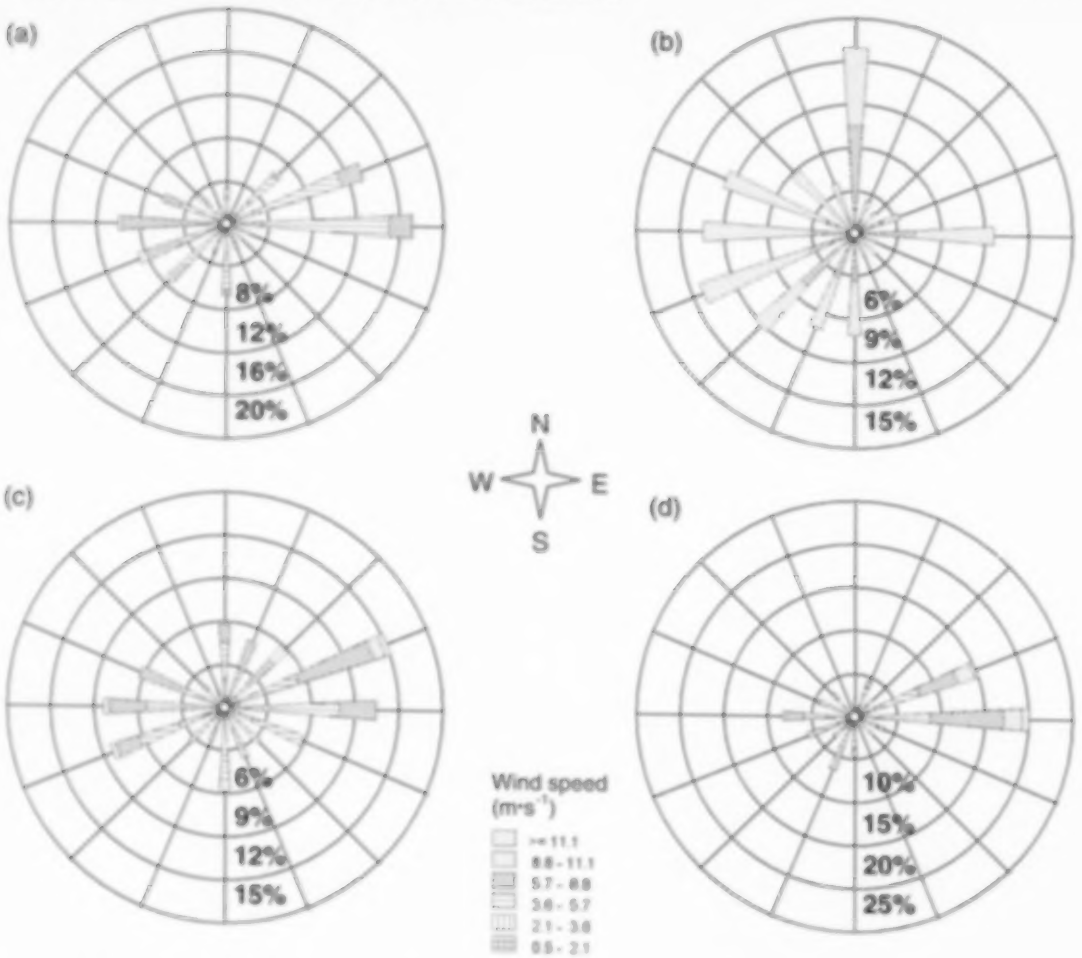
Fig. 3. Observed wind directions in 3 h intervals for western Lake Erie during the presumed walleye egg incubation period for (*a*) 1995, (*b*) 1996, (*c*) 1998, and (*d*) 1999. Bearings are as follows: 0° to 90° = NE; 90° to 180° = SE; 180° to 270° = SW; 270° to 360° = NW. The lines from bottom to top in each panel are 90°, 180°, and 270°.



of $< \sim 0.12$ m·s^{-1} is not. We will refer to this approximate threshold later in the text but recognize that further refinement of the concept would require extensive new laboratory and field studies.

During the first 5 days after peak hatching, the wind patterns again differed between the high and low recruitment years. For the two low recruitment years, winds from the north were predominant, while for the two high recruitment years, southwest winds were much more frequent (Fig. 5). Our simulations of the movement of positively buoyant par-

Fig. 4. Wind rose diagrams showing wind velocity ($m \cdot s^{-1}$) and vector (coming from) frequency (percentage) information during the presumed walleye incubation period in (*a*) 1995, (*b*) 1996, (*c*) 1998, and (*d*) 1999.



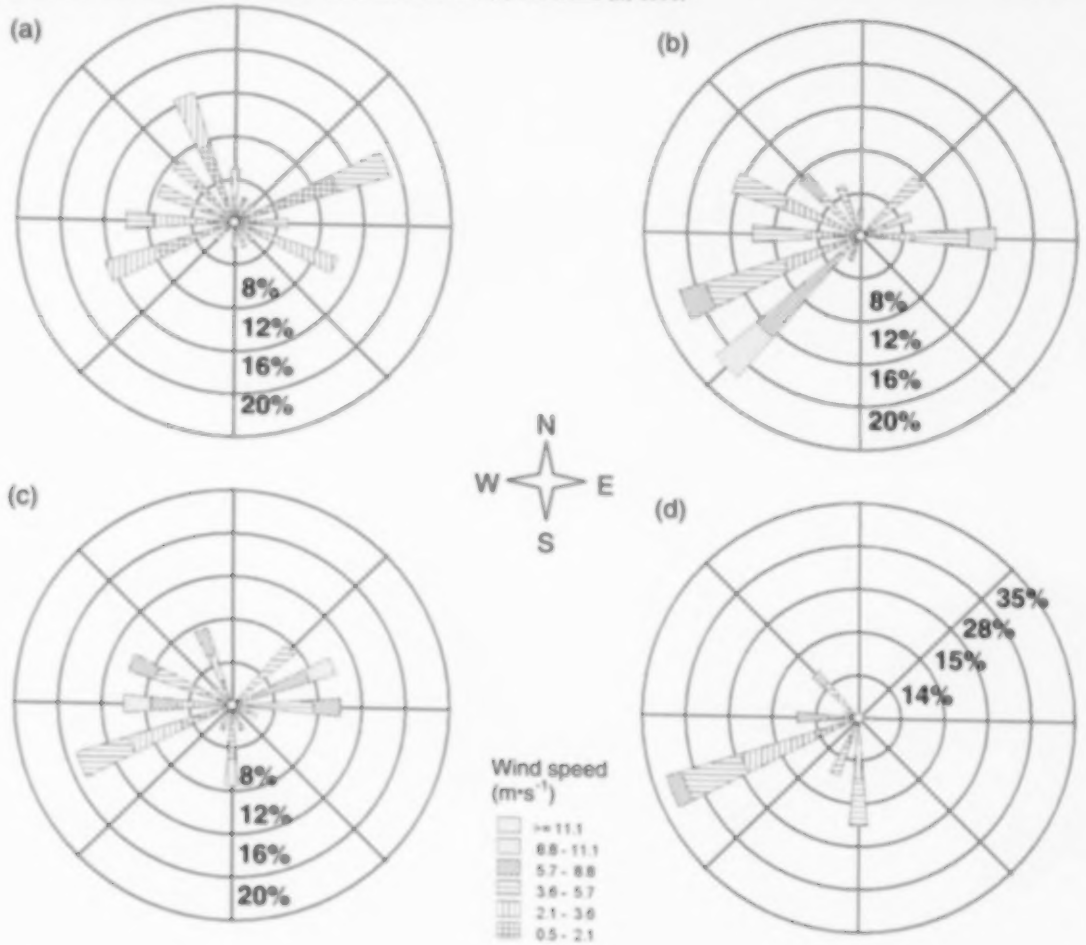
ticles released at the time of peak hatching in each year suggested that walleye larvae emerging at this time would be transported to very different areas by the lake surface currents acting in each year (Fig. 6). For the two high recruitment years (1996 and 1999), a large percentage of the particles ($> \sim 85\%$) ended up in the suitable nursery grounds. Conversely, for the two low recruitment years (1995 and 1998), only a relatively small ($< \sim 10\%$) percentage ended up in the suitable nursery areas. Further tracking of these particles for 7 and 10 days after hatch in each year showed that this distinction between years in particle distributions was maintained, although by day 10 in 1999, large numbers of larvae (assuming their swimming ability remains at the same level as that of newly hatched larvae) would be transported to inshore regions of the central basin.

Discussion

Walleye age-0 indices from fall trawling surveys are considered reliable predictors of age-2 walleye recruitment to fisheries (LEC–WTG 2006). This suggests that the weak relationship observed between spawning stock and age-2 walleye recruitment in Lake Erie is caused by density-independent agents of mortality operating within the first 6 months of the life of a cohort. Two of these agents are spring water warming rate and currents. The influence of spring warming rate on egg survival has been addressed by several researchers (Busch et al. 1975; Shuter and Koonce 1977; Roseman et al. 1999). These studies concluded that an increase in warming rate during incubation would shorten the incubation period and thus reduce the duration of exposure of eggs to various mortality agents.

However, there remains some variation in walleye recruitment unexplained by the warming rate. Specifically, spring warming rate does not explain the variation in recruitment observed among the 4 years in this study. Among the four recruitment years, the spring warming rate in 1998 is the highest, yet this year produced the second lowest recruitment. A slightly higher warming rate in 1999 than in 1995 (around 0.02 °C·day^{-1}) produced a 20-fold increase in re-

Fig. 5. Wind rose diagrams showing wind velocity ($m \cdot s^{-1}$) and vector (coming from) frequency (percentage) information during the 5-day period following the peak hatching date in (*a*) 1995, (*b*) 1996, (*c*) 1998, and (*d*) 1999.

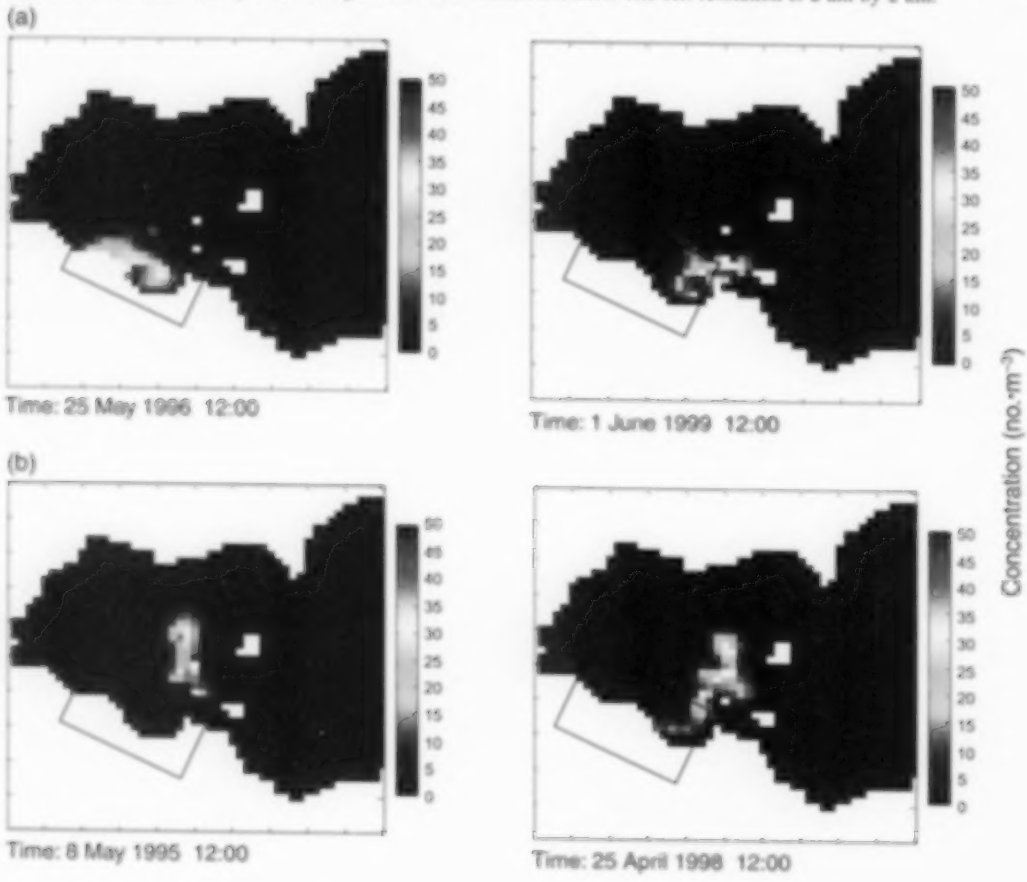


cruitment, far greater than would be predicted by the relationships of spring warming rate and fall walleye recruitment in Lake Erie (Roseman et al. 1999). For the 4 years considered in this study, water current differences among years appear to provide a better account of observed recruitment variation than spring warming rate alone.

Our study considered the influence of water currents on egg and larval survival. High water currents at walleye spawning grounds in the western basin can dislocate eggs from suitable hatching areas into muddy, unsuitable habitat and cause high mortality (Roseman et al. 2001). Our model predicted high current events during the early period of egg incubation in 1995 and 1998 (i.e., two low recruitment years). Comparing these two low recruitment years, we notice that the recruitment in 1998 is more than four times higher than that in 1995, although a similar magnitude of strong current were predicted for both years. Such variation may be a product of variations in warming rate and larval transportation patterns. Warming rate is slightly higher in 1998 than in 1995, and thus survivors from the extreme current events during egg incubation may have experienced higher survival than those in 1995. In addition, in 1998, a proportion (although small) of newly hatched larvae were able to reach suitable nursery ground. In contrast, almost all newly hatched larvae in 1995 were transported away from suitable nursery habitat. Accordingly, we would predict the lowest recruitment in 1995 from our modeling. This is consistent with field observations. High current events were not predicted in 1996 and 1999, when recruitment was much higher. High currents were predicted later in the spawning period in 1999, but we speculate that this may have been after the majority of deposited eggs had completed incubation. In addition to a higher spring warming rate compared with 1999, almost all newly hatched larvae were transported to the suitable nursery ground in 1996. Therefore, both favourable water temperatures and water currents could have combined to produce the highest walleye recruitment levels in 1996. Again, this is consistent with field observations.

At shallow walleye spawning grounds in western Lake Erie, the strength of bottom currents depends on both wind

Fig. 6. Simulated concentrations of positively buoyant particles 5 days after the peak hatching date for two high (*a*, top two panels) and two low (*b*, bottom two panels) recruitment years. The rectangle indicates the approximate area of suitable nursery habitat. High concentrations are colored orange and red; low concentrations are blue. The pink star shows the walleye spawning grounds (i.e., area 5 in Fig. 1) where larvae were released at the observed peak hatching time in the simulation studies. The cell resolution is 2 km by 2 km.



speed and wind direction. Moderate northeast winds combine with long fetches to transfer energy from wind to water mass, producing high water currents at the downwind side of the lake, where the spawning reefs are located. In addition, the complex topographic features of spawning reefs (shallow, uneven, and close to the coast) can break waves (i.e., creating strong wave surge) coming from the directions that winds blow, thus producing strong turbulence at the spawning grounds.

Our model results also suggest that interannual differences in current patterns in the posthatch period are important in determining differences in recruitment success. Winds from the west predominated in the years of high recruitment. In the Northern Hemisphere, the rotation of the earth causes the direction of motion of wind-driven particles and fluids (e.g., water mass movement) to deflect to the right of the direction of the wind that is driving them (i.e., Coriolis effect and Ekman transportation). This deflection is expected for water currents and is both observed in Lake Erie (Gedney and Lick 1972) and simulated by ELCOM. Thus, we expect westerly winds in western Lake Erie to produce a generally southerly (toward the southern boundary of the basin) movement of water mass over the walleye spawning grounds, and this movement favours a timely transport of larval walleye to the south shore nursery grounds.

The ideas of "critical periods" (Hjort 1914) and "match/mismatch" (Cushing 1990), wherein fish larvae need to reach suitable habitats for survival and growth before starvation, has been thoroughly investigated in marine systems (Brickman and Frank 2000; Zhao et al. 2001; Hinrichsen et al. 2003). Fechhelm and Fissel (1988) and Fechhelm and Griffiths (1990) observed that the recruitment of Canadian Arctic cisco (*Coregonus autumnalis*) in Alaskan waters was significantly related to summer easterly wind. However, this hypothesis has rarely been examined in detail in freshwater systems. Using a simple biophysical model, Jones et al. (2003) demonstrated that the survival of newly hatching larvae incubated in the Sandusky River depended on the time (or distance) that the larvae were transported from riverine spawning habitats to lacustrine (Sandusky Bay of Lake Erie) nursery grounds. Similarly, coupled physical–biological models have been developed to examine these fish recruit-

ment processes for marine species (Miller 2007), but we know of no other published studies that have used this approach in large lakes.

The prevailing wind patterns during spring in Lake Erie may explain why the midlake reef complex is such an important walleye spawning ground in Lake Erie. During this time, westerly winds are predominant, which our model suggests provide ideal conditions for transportation of recently hatched larval walleye to ideal nursery habitat along the Lake Erie southern shore. As well, winds from the west and especially the southwest experience a short fetch, limiting their ability to create damaging currents in these spawning areas. It seems to be a general pattern that the other regions of the Great Lakes where walleye production is large and sustainable are all areas with comparatively small fetches, relative to the main lake areas (e.g., Bay of Quinte of Lake Ontario, Lake St. Clair, Saginaw Bay, and eastern Georgian Bay of Lake Huron).

The modeling results presented here have a number of important limitations that will motivate the next steps in our research program. First, the model does not provide a mechanistic connection between physical hydrodynamic processes in western Lake Erie and walleye early life survival processes. For example, if the model included an egg hatching schedule that is temperature-dependent, it would permit realistic simulations of differences in peak hatching time across years. Similarly, accumulated growing degree days is a good predictor for the time when fish larvae start first feeding (Zhao et al. 2001), and thus adding such a function to the model would permit more realistic simulations of vulnerability times rather than imposing a fixed (5 days) period of vulnerability. Linking an individual-based model to the hydrodynamic models is an alternative approach to incorporate biological components, such as growth rate and time to first feeding. In addition, a shear stress model of current magnitude effects on egg dislocation would provide a more accurate assessment of whether strong currents are likely to have affected recruitment in the low recruitment years.

Periods of egg incubation and peak hatching were selected from continuous time series of egg and larval abundance derived from field data collected by Roseman (2000) and Roseman et al. (2001, 2005). Because early life history events occur rapidly for walleye (McElman and Balon 1979), attempts were made to sample eggs twice weekly and pelagic larvae at least once per week; however, equipment reliability and weather conditions sometimes dictated when sampling occurred. According to Roseman et al. (2005), the sampling interval was prolonged between pelagic larval samples in 1996 and 1999, possibly resulting in missing the period when peak pelagic larval abundance occurred. Therefore, the timing of true peak larval abundance could have occurred earlier in May than reported here.

Although it is recognized that the midlake reef complex is the largest spawning ground in the lake and that recruitment from this complex is probably the major source of walleye recruitment for the lake, recruitment in the west basin of Lake Erie may come from multiple sources, including several individual reefs in the midlake complex, three major tributaries (i.e., Maumee and possibly Sandusky rivers — Mion et al. 1998; Detroit River — Manny et al. 2007), and some smaller spawning grounds in the northern part of the basin. Schedules for spawning and hatching, as well as environmental conditions (such as temperature and water currents), likely vary among the different spawning areas, and therefore there is likely to be considerable interannual variation in the relative contribution of spawning sites to overall walleye annual recruitment. Developing a more mechanistic picture of how reproductive success varies across these spawning locations would provide a valuable extension of our understanding of the sources of recruitment variation in Lake Erie walleye.

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Differences in predicted catch composition between two widely used catch equation formulations

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Abstract: Fishing gear selectivity varies among different types of fish (e.g., species, age, sex, or length groups), but their relative catch composition also depends on the fishing process. The continuous (Baranov) formulation assumes that fishing mortality and natural mortality occur together during the fishing season and that there are multiple encounters between fish and fishing gear. For this formulation, predicted catch composition depends on fishing mortality, and at high fishing mortality levels the entire population can be caught provided the selectivity is nonzero for all age groups. In contrast, the discrete formulation assumes that fishing mortality occurs separately from natural mortality and that fish encounter at most only one set of fishing gear. The discrete formulation is easier to compute, but the predicted catch composition is independent of fishing mortality, and some of the population remains unexploitable. The correct choice of equations depends on the particular fishery and fishing mortality levels; at low fishing mortality levels the predictions differ little, but at high fishing mortality levels where multiple gear encounters could occur, the continuous formulation is preferable.

Résumé : La sélectivité des engins de pêche varie en fonction des divers types de poissons (d'après l'espèce, l'âge, le sexe ou la classe de taille), mais la composition relative de la récolte dépend aussi du processus de pêche. La formulation continue (de Baranov) présuppose que la mortalité due à la pêche et la mortalité naturelle vont de pair durant la saison de la pêche et qu'il y a des rencontres multiples entre les poissons et les engins de pêche. Dans cette formulation, la composition de la capture prédite dépend de la mortalité due à la pêche et, aux taux élevés de mortalité due à la pêche, toute la population peut être capturée à condition que la sélectivité ne soit égale à zéro pour aucune des classes d'âge. En contraste, la formulation discontinue présuppose que la mortalité due à la pêche peut survenir séparément de la mortalité naturelle et que les poissons rencontrent au plus une série d'engins de pêche. La formulation discontinue est plus facile à calculer, mais la composition de la capture prédite est indépendante de la mortalité due à la pêche et une partie de la population reste à l'abri de l'exploitation. Le bon choix d'équations dépend de la pêche en question et de l'importance de la mortalité due à la pêche; aux faibles taux de mortalité due à la pêche, il y a peu de différence entre les prédictions, mais aux forts taux de mortalité due à la pêche lorsqu'il peut se faire des rencontres multiples d'engins de pêche, la formulation continue est préférable.

[Traduit par la Rédaction]

Introduction

All types of fishing methods are selective to some extent (for species, sex, length, and age), and fisheries stock assessments must account for selectivity to accurately predict the effects of fishing on a population, particularly when fitting models to age and length frequency data. In some fisheries, estimates of selectivity are available from mark–recapture data (Myers and Hoenig 1997; Clark and Kaimmer 2006) or can be obtained experimentally (Millar and Fryer 1999), but often the selectivity parameters must be estimated within stock assessment models (e.g., Megrey 1989).

The most commonly used stock assessment models are age-structured, but not all account for potential interactions between selectivity and fishing mortality. As fishing mortality increases, the probability of encountering the fishing gear increases too, and at the highest levels, even fish with low gear selectivity will eventually be caught. Conceptually, imagine fish migrating through a gauntlet of successive fishing nets. When fishing pressure is low, fish will generally encounter at most one net, and the catch composition will in essence be a population sample modified by gear selectivity. But when fishing pressure is high, most fish will encounter a net, and fish with high selectivity will be caught by the first net, while those with low selectivity will survive but then will encounter another net. If they survive that second net, they may still encounter another net and so on. Under this scenario, even the fish with low selectivity will eventually be caught, and the catch composition will differ from that obtained under conditions of low fishing mortality.

Given this conceptual model, it makes sense that fishery stock assessment models should include this interaction between fishing mortality and selectivity when predicting catch compositions. However, in practice only one of two widely used formulations, the continuous formulation, includes this interaction, while the discrete formulation predicts that catch composition remains constant regardless of fishing mortality. The discrete formulation assumes that

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fishing occurs in a single pulse and can be separated from natural mortality, whereas the continuous formulation assumes that natural mortality and fishing mortality occur simultaneously. More subtly, the discrete formulation assumes that there is at most one interaction between fish and fishing gear, whereas the continuous formulation assumes that there are many potential interactions between fish and fishing gear.

The historical origins of these two formulations are outlined below, before examining their differing predictions for catch and population composition. The following differences are highlighted: (*i*) the discrete case predicts the same catch composition regardless of fishing intensity, whereas the continuous case predicts that catch composition will vary with fishing intensity; and (*ii*) the discrete model generally implies that a portion of the population is unexploitable, whereas the continuous model allows the entire population present at the start of the fishing season to be caught.

Continuous formulation of selectivity

The continuous formulation for fisheries models can be traced back to Baranov (1918), derived under the assumptions that fishing mortality (F) and natural mortality (M) are proportional to fish abundance (N) and act simultaneously and uniformly throughout the year, i.e., $dN/dT = -(M + F)N$. The solution to this differential equation yields the classic Baranov catch equations (Beverton and Holt 1957; Ricker 1975; Hilborn and Walters 1992):

$$(1) \qquad N_{t+1} = N_t e^{-M-F}$$

$$(2) \qquad C_t = \frac{F}{M+F} N_t e^{-M-F}$$

where t is time, M is the instantaneous natural mortality rate, N_t is fish abundance, F is the instantaneous fishing mortality rate, and C_t is the observed catch. To allow for annual changes in fishing mortality, F in eqs. 1–2 can be replaced with F_t to denote the fishing mortality in year t.

This formulation can be applied to fish populations that are divided into groups of fish such as age, sex, or length groups; for ease of presentation, the groups are treated as age groups. Tracking fish numbers at each age would in theory require age-specific natural mortality and fishing mortality, but this would result in more parameters to estimate than available data. To simplify the model, it is nearly always assumed that natural mortality is constant for all age groups and that fishing mortality can be separated into age-specific gear selectivity (S_a) and year-specific fishing intensity. This idea of separable fishing mortality, written $F_{a,t} = S_a F_t$, is sometimes attributed to Doubleday (1976), but actually originated in an earlier unpublished manuscript, Pope (1974), as acknowledged by Doubleday. With the addition of age structure, constant natural mortality, and separable selectivity, the Baranov catch equations are converted to

$$(3) \qquad N_{a+1,t+1} = N_{a,t} e^{-M-S_a F_t}, \quad S_a \geq 0$$

$$(4) \qquad C_{a,t} = \frac{S_a F_t}{M + S_a F_t} N_{a,t} e^{-M-S_a F_t}$$

where a is the age group and S_a is gear selectivity. To ensure that both fishing mortality and selectivity are estimable, the relative selectivity parameters need to be constrained in some way, for example by setting selectivity to one for a prominent age group (Pope and Shepherd 1982).

This basic set of equations can be modified in variety of ways. For example, the number of parameters can be reduced by assuming that selectivity is a smooth function of age (Fournier and Archibald 1982), model complexity can be increased by assuming a random walk in selectivity over time (Butterworth et al. 2003), and the equations can be extended to incorporate seasonal variability in fishing effort (MacCall 1986; Mertz and Myers 1996).

One of the key features of the continuous formulation is that selectivity and fishing mortality are exponentiated, which implies that at infinite fishing mortality levels all fish can be caught. This paper does not explore the implications that there are actually some age groups that are not available (e.g., in habitat that the fishing gear cannot access), although eqs. 3–4 can be modified to include age-specific availability, A_a (Megrey 1989):

$$(5) \qquad N_{a+1,t+1} = N_{a,t} A_a e^{-M-S_a F_t} + N_{a,t}(1 - A_a) e^{-M}, \quad 0 \leq A_a \leq 1$$

$$(6) \qquad C_{a,t} = \frac{S_a F_t}{M + S_a F_t} A_a N_{a,t} e^{-M-S_a F_t}$$

A long list of papers and stock assessment models have incorporated the continuous formulation at their heart (e.g., Megrey 1989), from Doubleday (1976) to Fournier and Archibald (1982), to the Cagean (Deriso et al. 1985) and Stock Synthesis models (Methot 1989), and finally to more modern statistical catch-at-age models (e.g., Punt et al. 2001; Butterworth et al. 2003).

Discrete formulation of selectivity

The continuous formulation assumes that fishing mortality and natural mortality occur together. If instead, fishing occurs in a very short pulse and no natural mortality occurs during the fishing season, then the discrete formulation is often employed. For the discrete formulation, the fishing season could be at the start of the year, the end of the year, or at any point during the year (e.g., Ricker 1944; Quinn and Deriso 1999). If the fishing season occurs in an instant at time, t^*, then the relevant equations (including the separability assumption) are

$$(7) \qquad N_{a+1,t+1} = N_{a,t}(1 - S_a u_t) e^{-M}, \quad 0 \leq u_t \leq 1, \quad 0 \leq S_a \leq 1$$

$$(8) \qquad C_{a,t} = N_{a,t} S_a u_t e^{-(t^* - t)M}$$

where u_t is the annual exploitation rate for fully selected ages. Note that u_t in the discrete case and F_t in the continuous case are related; in the absence of selectivity and natural mortality, $u_t = 1 - e^{-F_t}$. The key difference from the continuous formulation is that selectivity is not exponentiated together with fishing mortality. One great advantage of the discrete formulation is that it is easy to solve for u_t, whereas the Baranov equations must be solved numerically.

The most widely used version of the discrete model

comes from "cohort analysis" (Pope 1972), where it was presented as an approximation to the Baranov catch equations. The Pope approximation is typically written $N_{a,t} = C_{a,t}e^{0.5M} + N_{a+1,t+1}e^{M}$ because cohort analysis works backward from current population numbers, but can be rewritten as $N_{a+1,t+1} = (N_{a,t}e^{-0.5M} - C_{a,t})e^{-0.5M}$ (i.e., the discrete formulation with $t^* = 0.5$ in eqs. 7–8). This approximation diverges from the Baranov equations at high mortality levels, and several improvements are possible (e.g., Ulltang 1977; Sims 1982; MacCall 1986). For example, Allen and Hearn (1989) demonstrate that $N_{a+1} = N_ae^{-M} - C_ae^{-0.585M}$ predicts N_{a+1} to within 1% for total mortality less than 1.5. Nevertheless, the Pope approximation remains in wide usage because it is not only an approximation to the Baranov equations, but also a correct model of a pulse fishery operating in the exact middle of the year, while the alternative approximations are not quite as easily interpreted.

The Pope approximation is used in a number of stock assessment methods and programs: cohort analysis (Pope 1972), ADAPT (Gavaris 1988), extended survivors analysis (Shepherd 1999), Coleraine (Hilborn et al. 2003), as one option in Stock Synthesis II (Methot 2005), and in a variety of other stock assessments (Punt and Walker 1998; Parma 2002; Punt 2003). The discrete formulation is also present in the delay-difference model of Deriso (1980) and in the form where all fishing occurs in a pulse at the start of the year (i.e., $t^* = t$) in Punt and Kennedy (1997).

Materials and methods

Having established the history behind the two formulations, I examine the predicted age compositions for the discrete formulation, derive the continuous formulation from the assumption that there are multiple gear encounters, generalize the continuous formulation to a fishing season of arbitrary length within the year, and use a generic example to contrast the predicted catch and population compositions between the continuous formulation and the discrete formulation.

Predicted age composition for the discrete formulation

For the discrete formulation with the fishing season occurring at any time t^* during the year (eqs. 7–8), the predicted population composition at the end of the year, $A^*_{a,t}$, and catch composition, $A^c_{a,t}$, are

$$(9)\qquad A^*_{a,t} = \frac{N_{a,t}(1 - S_a u_t)e^{-M}}{\sum_a N_{a,t}(1 - S_a u_t)e^{-M}} = \frac{N_{a,t}(1 - S_a u_t)}{\sum_a N_{a,t}(1 - S_a u_t)}$$

$$(10)\qquad A^c_{a,t} = \frac{S_a u_t N_{a,t}e^{-(t^* - t)M}}{\sum_a S_a u_t N_{a,t}e^{-(t^* - t)M}} = \frac{S_a N_{a,t}}{\sum_a S_a N_{a,t}}$$

Note that the predicted catch composition does not depend on exploitation rate or natural mortality. It depends only on selectivity and the abundance of fish in each group. Furthermore, the predicted catch composition does not depend on the timing of the fishery; the fishery could operate at the beginning, in the middle, or at the end of the year.

The discrete formulation assumes that fish interact at most once with the fishing gear each year. If they escape the encounter (low selectivity), they will not encounter another set of fishing gear in the same year. There are many fisheries for which this is a perfectly reasonable assumption, but in some short and intense terminal fisheries, migrating fish are forced to run a gauntlet of fishing gear. In such fisheries, catch composition will be influenced by fishing mortality, leading naturally to the derivation of the continuous formulation for pulse fisheries presented in the next section.

Deriving the continuous formulation of selectivity

Consider the case of a pulse fishery that occurs over a short enough period of time (Δt) to ignore natural mortality. If F_t is defined as the fraction of the fully selected population that would be caught by m gear encounters, then the probability of age group a not being caught during a single gear encounter is $1 - \frac{S_a F_t}{m}$. This must be raised to the power of m to reflect that there are m encounters with the fishing gear, resulting in the following equations:

$$(11)\qquad N_{a+\Delta t,t+\Delta t} = N_{a,t}\left(1 - \frac{S_a F_t}{m}\right)^m$$

$$(12)\qquad C_{a,t} = N_{a,t}\left[1 - \left(1 - \frac{S_a F_t}{m}\right)^m\right]$$

For general use, these equations are impractical, since they are awkward to solve, and it is unlikely that the exact number of gear encounters would be known, but as the number of gear encounters increases ($m \to \infty$), they simplify to

$$(13)\qquad N_{a+\Delta t,t+\Delta t} = N_{a,t}e^{-S_a F_t}$$

$$(14)\qquad C_{a,t} = N_{a,t}\left(1 - e^{-S_a F_t}\right)$$

$$(15)\qquad A^c_{a,t} = \frac{N_{a,t}\left(1 - e^{-S_a F_t}\right)}{\sum_a N_{a,t}\left(1 - e^{-S_a F_t}\right)}$$

In other words, even though the fishery occurs over a very short interval, the predicted catch composition depends on fishing mortality, unlike for the discrete formulation. Note that eqs. 13–14 are similar to the depletion model popularized by DeLury (1947), but also include age-varying selectivity in the form presented in Paloheimo (1980).

Generalizing the continuous formulation

The derivation in eqs. 13–15 excludes natural mortality, but if natural mortality and fishing mortality co-occur then the portion of natural mortality within the fishing interval Δt is $M\Delta t$, and the Baranov equations (eqs 3–4) apply with M replaced by $M\Delta t$ and F replaced by $F\Delta t$.

To generalize the equations to a fishing season of any duration, consider the year to be defined from time t to $t + 1$, while the fishing season operates within the year from time t_1 to t_2 (the duration is $\Delta t = t_2 - t_1$). Natural mortality will operate alone during the intervals $[t, t_1]$ and $[t_2, t + 1]$ and

simultaneously with fishing mortality during the interval $[t_1, t_2]$. The resulting equations including natural mortality and generalized for a fishing season of any length are

$$(16)\quad N_{a+1,t+1} = N_{a,t}\left[e^{-M(t_1-t)}\right]\left[e^{-(S_aF_t+M)\Delta t}\right]\left[e^{-M(t+1-t_2)}\right] = N_{a,t}e^{-S_aF_t\Delta t - M}$$

$$(17)\quad C_{a,t} = \frac{S_aF_t}{S_aF_t+M}N_{a,t}e^{-M(t_1-t)}\left[1-e^{-(S_aF_t+M)\Delta t}\right]$$

$$(18)\quad A^c_{a,t} = \frac{N_{a,t}e^{-S_aF_t\Delta t}}{\sum_a N_{a,t}e^{-S_aF_t\Delta t}}$$

$$(19)\quad A^c_{a,t} = \frac{\frac{S_a}{S_aF_t+M}N_{a,t}\left[1-e^{-(S_aF_t+M)\Delta t}\right]}{\sum_a \frac{S_a}{S_aF_t+M}N_{a,t}\left[1-e^{-(S_aF_t+M)\Delta t}\right]}$$

Note that eq. 17 must be solved to find the value of F_t that produces the observed catch. As the fishing interval Δt tends to zero, the estimated value of F_t increases towards infinity, which may result in numerical difficulties when solving for F_t. However, the product $F'_t = F_t\Delta t$ tends to a constant value as Δt tends to zero; therefore, it may be easier to solve these equations if F_t is replaced by F'_t.

There are several interesting special cases of the predicted catch composition (eq. 19). When there is a very short pulse fishery (i.e., $\Delta t \to 0$ while the observed catch remains constant) but multiple gear interactions occur, the predicted catch composition does not depend on natural mortality and is of a similar form to eq. 15 above:

$$(20)\quad \lim_{\substack{\Delta t \to 0\\ F_t \to \infty,\\ \text{subject to}\\ F_t\Delta t \to F'_t}} A^c_{a,t} = \frac{N_{a,t}\left(1-e^{-S_aF'_t}\right)}{\sum_a N_{a,t}\left(1-e^{-S_aF'_t}\right)}$$

Under conditions of low fishing mortality (as $F_t \to 0$), the predicted catch composition will be identical to that predicted by the discrete formulation (eq. 10), which makes sense because the probability of encountering the fishing gear more than once becomes vanishingly small when there is little fishing:

$$(21)\quad \lim_{F_t \to 0} A^c_{a,t} = \frac{S_aN_{a,t}}{\sum_a S_aN_{a,t}}$$

When fishing mortality is very high, natural mortality and selectivity both become irrelevant (provided $S_a > 0$ for all a), and the near-census catch composition becomes identical to the population composition at the start of the season.

$$(22)\quad \lim_{F_t \to \infty} A^c_{a,t} = \frac{N_{a,t}}{\sum_a N_{a,t}}$$

Finally, for year-round fishing ($\Delta t = 1$), the prediction is the same as for the standard Baranov catch equations:

$$(23)\quad \lim_{\Delta t \to 1} A^c_{a,t} = \frac{\frac{S_a}{S_aF_t+M}N_{a,t}\left(1-e^{-S_aF_t-M}\right)}{\sum_a \frac{S_a}{S_aF_t+M}N_{a,t}\left(1-e^{-S_aF_t-M}\right)}$$

Maximum possible catch for discrete and continuous formulations

The two formulations imply quite different levels of maximum catch. Under the discrete formulation, it is not possible for the fishery to catch the entire population at the start of the fishing period unless $S_a = 1$ for all a. The maximum catch is

$$(24)\quad C^{max}_t = \sum_a N_{a,t}S_ae^{-(t'-t)M}$$

For the continuous formulation, the entire population present at the start of the fishing interval can be caught (provided all $S_a > 0$), except for fish that died from natural mortality between the start of the year and the start of fishing:

$$(25)\quad C^{max}_t = \sum_a N_{a,t}e^{-(t_1-t)M}$$

Example

To illustrate the differences between the discrete and continuous formulations of selectivity, a model is presented of a single fishing year. The population at the start of the year was assumed to number $N_{total} = 1000$ fish from ages $a = 1$ to 10 (no plus group) and equilibrium numbers at age obtained by assuming a stable age distribution with $M = 0.1$. The initial population structure prior to fishing was

$$(26)\quad N_1 = N_{total}\frac{1-e^{-M}}{1-e^{-10M}}$$
$$N_{a+1} = N_ae^{-M}$$

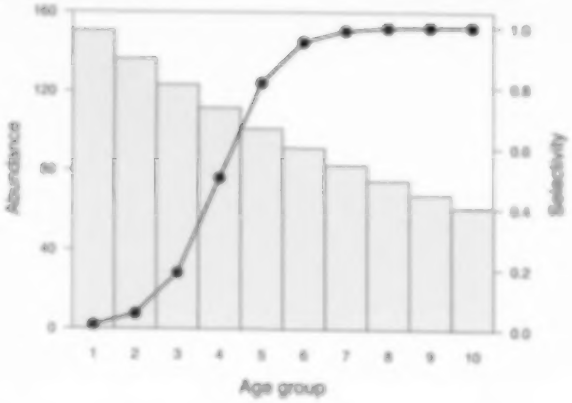
The selectivity of the fishing gear was assumed to be logistic, with 50% retention (a_{50}) at age 4 and 95% selectivity (a_{95}) at age 6 (e.g., Punt et al. 2001):

$$(27)\quad S_a = \left[1+\exp\left(-\ln 19\frac{a-a_{50}}{a_{95}-a_{50}}\right)\right]^{-1}$$

The age structure and selectivity of this simulated population are illustrated (Fig. 1). Note that selectivity is 0.01 for age 1 and 0.99 or greater for ages 8–10.

The predicted population composition and catch composition are calculated as a function of different catch levels (as a fraction of N_{total}) from 0.001 to the maximum possible for each formulation, with $M = 0.1$. Five scenarios are produced: (*a*) discrete start: discrete formulation with fishing at the start of the year (eqs. 9–10); (*b*) discrete middle: discrete formulation with pulse fishing in the exact middle of the year (eqs. 9–10) (i.e., the Pope approximation); (*c*) continuous half-season: continuous formulation, fishing season is in the first half of the year (eqs. 18–19); (*d*) continuous middle: continuous formulation with pulse fishing in the exact middle of the year (eqs. 18, 20); (*e*) continuous year-round: continuous formulation, fishing

Fig. 1. Number at age (bars) and selectivity (circles) of the simulated population.



is throughout the year (eqs. 18, 24) (i.e., the standard Baranov equations).

Results

Predicted age compositions from the discrete and continuous formulations were similar at low catch levels but differed greatly at high catch levels (Fig. 2). For the discrete formulation, predicted catch composition was constant over all catch levels, with negligible catch proportions of age groups 1 and 2; whereas for the continuous formulation, the catch proportions of lower age groups increased with increasing catches, and when the catch level approached the prefishery population size (i.e., the catch became near-census of the population), age 1 and age 2 fish were the most common age groups in the catch (Fig. 2). The other major difference was that maximum possible catches were only about 50% of the population for the discrete formulation (Figs. 2*a*–2*b*), but close to 100% for all of the continuous formulation scenarios (Figs. 2*c*–2*e*).

Predicted age compositions were nearly identical for the two discrete scenarios, but the maximum possible catch was lower when catches were modeled in the middle of the year (instead of at the start of the year), because some of the fish died of natural mortality before the fishing season started.

For the continuous formulation, predicted age compositions were similar for all three scenarios, except that when the fishing season was a short pulse in the middle of the year, it was not possible to catch the entire population because of natural mortality reducing the population prior to the fishing season. There was little difference in predicted age composition between the other two scenarios (fishing over the first half of the year vs. fishing throughout the year), although these differences would have increased if natural mortality had been assumed to be higher than the 0.1 used in the simulations.

Discussion

Fisheries differ greatly in their intensity, degree of selectivity, and season length within a year; these factors should be taken into account when deciding which equations best model the fishing process. In reality, however, the great majority of age-structured models currently in use either incorporate the continuous formulation (Baranov 1918) or the Pope (1972) version of the discrete formulation. These two classes of catch equations make different assumptions about the fishing process, and as fishing mortality increases, their predicted catch compositions diverge.

The underlying premise of the continuous formulation is that fishing mortality and natural mortality occur simultaneously during the fishing season and that at high fishing mortality levels, even fish with low selectivity (provided this is nonzero) will eventually be caught. This is analogous to fish running a gauntlet of nets; given enough nets, even fish with low selectivity will eventually be caught. If some groups of fish cannot be caught because they do not inhabit the region where fishing occurs, then age-specific availability should be included in the equations, as in eqs. 5–6.

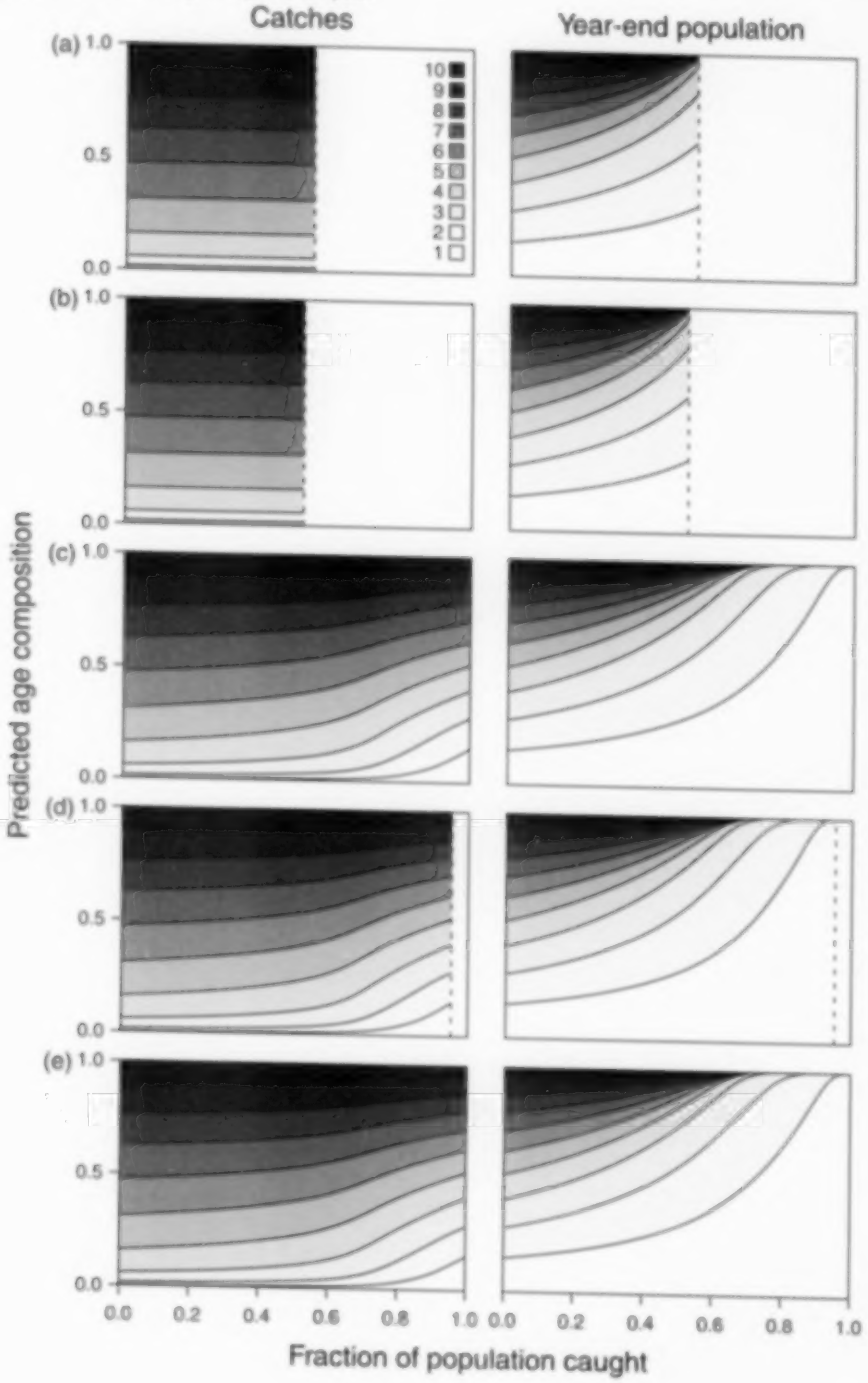
In contrast, the discrete formulation assumes that fishing mortality and natural mortality occur in consecutive or interleaved periods of time and that levels of fishing mortality do not affect catch age composition. The maximum possible catch is constrained by selectivity; unless all fish have equal selectivity, it is not possible to catch the entire stock. In a sense, the selectivity parameters in the discrete formulation are actually availability parameters, since low selectivity groups will contain unexploitable fish.

The most appropriate choice of equations depends on the circumstances of the fishery, the desired accuracy of the model, and the need for speed in model execution. If fish could encounter the fishing gear at most once, then the discrete case is more appropriate. There are only a few scenarios where fish could encounter the fishing gear once, such as a single net strung across a river to catch migrating fish or a very short duration but widespread fishery. In many fisheries, however, fish escaping from one gear encounter will not be free from further encounters.

If multiple gear encounters are possible but fishing mortality is low, the actual number of gear encounters in practice would also be low, and the discrete and continuous formulations would provide nearly identical predictions. A similar situation would arise when selectivity does not differ among age groups. Under these scenarios, although the continuous case describes the fishery process better, some modelers may prefer to use the linear equation of the discrete case because the harvest rate u_i can be obtained directly, whereas the nonlinear equation of the continuous case requires a minimization step to estimate fishing mortality F_i. In complex models, considerable time may be saved by omitting the estimation step required for the continuous formulation.

Finally, where multiple gear encounters are possible and fishing mortality is potentially high, the continuous case should be preferred because it more realistically predicts the resulting catch composition. This holds true even if the actual fishing season is of short duration. A classic example falling into this category is the fishery for sockeye salmon (*Oncorhynchus nerka*) in parts of Bristol Bay, Alaska, where the fishing season is short (2–3 weeks), catches commonly exceed 50% of the returning fish, and selectivity differs among the various age classes of sockeye salmon.

Fig. 2. Predicted catch (left column) and population (right column) age composition given different levels of catch for the simulated population represented in Fig. 1, under the discrete formulation (*a–b*) and continuous formulation (*c–e*). Gray shading represents the different age groups from 0 to 10, while broken lines in some plots represent maximum possible catch levels. The five scenarios depicted are (*a*) discrete formulation with fishing at the start of the year, (*b*) discrete formulation with pulse fishing in the exact middle of the year, (*c*) continuous formulation with fishing in the first half of the year, (*d*) continuous formulation with pulse fishing in the exact middle of the year, and (*e*) continuous formulation with fishing throughout the year.



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Competition between native and introduced salmonid fishes: cutthroat trout have lower growth rate in the presence of cutthroat–rainbow trout hybrids

Steven M. Seiler and Ernest R. Keeley

Abstract: When nonnative species become established within new communities, competition may play a role in determining the persistence of ecologically similar native species. In western North America, many native cutthroat trout (*Oncorhynchus clarkii*) populations have been replaced by nonnative rainbow trout (*Oncorhynchus mykiss*). Superior competitive ability of rainbow trout and cutthroat–rainbow trout hybrids is often cited for this replacement; however, few studies have tested for mechanisms that might allow introduced rainbow trout to out-compete native trout species. Our previous work found individual-based differences in swimming and foraging ability among cutthroat trout, rainbow trout, and their hybrids. In this study, we tested for the presence and strength of competition between cohorts of cutthroat trout, rainbow trout, and their reciprocal hybrids. We assayed the growth rate of juvenile cutthroat trout in allopatry versus cutthroat trout when sympatric with rainbow trout and each hybrid cross. After controlling for size and density of trout, cutthroat trout cohorts in stream channels that contained hybrid genotypes experienced lower growth than cutthroat trout in allopatry. Averaged across heterospecific treatments, cutthroat trout growth was also lower than that of cutthroat trout cohorts in allopatry. Our study suggests that juvenile cutthroat trout experience a growth disadvantage when competing against cutthroat–rainbow hybrids.

Résumé : Lorsque des espèces non indigènes se sont établies dans de nouvelles communautés, la compétition peut jouer un rôle dans la détermination de la persistance des espèces indigènes à écologie semblable. Dans l'ouest de l'Amérique du Nord, plusieurs populations de la truite fardée (*Oncorhynchus clarkii*) indigène ont été remplacées par la truite arc-en-ciel (*Oncorhynchus mykiss*) non indigène. Pour expliquer cette substitution, on évoque souvent la compétitivité supérieure des truites arc-en-ciel et des hybrides de la truite arc-en-ciel et de la truite fardée; cependant, peu d'études ont cherché à vérifier les mécanismes qui pourraient permettre à la truite arc-en-ciel de gagner la compétition avec les espèces indigènes de truites. Notre recherche antérieure a révélé des différences basées sur l'individu dans la capacité de nage et de recherche de nourriture entre la truite fardée, la truite arc-en-ciel et leurs hybrides. Dans le présent travail, nous cherchons à démontrer la présence et la force de la compétition entre les cohortes de truites fardées, de truites arc-en-ciel et de leurs hybrides réciproques. Nous avons mesuré le taux de croissance de jeunes truites fardées en conditions d'allopatrie ainsi que dans des conditions de sympatrie avec la truite arc-en-ciel et avec chacun des croisements hybrides. Après avoir tenu compte de la taille et de la densité des truites, nous observons que les cohortes de truites fardées dans les cours d'eau contenant des génotypes hybrides ont une croissance plus faible que les truites fardées en conditions d'allopatrie. En moyenne dans toutes les conditions expérimentales hétérospécifiques, la croissance des truites fardées est aussi plus faible que celles des cohortes de truites fardées en conditions d'allopatrie. Notre étude indique que les jeunes truites fardées subissent un désavantage dans leur croissance lorsqu'elles sont en compétition avec des hybrides de la truite fardée et de la truite arc-en-ciel.

[Traduit par la Rédaction]

Introduction

The role of competition, predation, and abiotic factors in determining community structure has received much attention from ecologists (Hairston et al. 1960; Strong et al. 1986). In natural communities, ecologically similar species are thought to coevolve to limit energetically costly competitive interactions (Brown 1971; Simberloff 1981). In contrast, the introduction of nonnative species forces sympatry between taxa that have not coevolved. When such introductions bring together ecologically similar native and nonnative species, competition for resources is likely very strong

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(Fausch 1988; Petren and Case 1996). The pattern of native population declines or extinctions after the introduction of a similar species provides observational support that introduced species experience competitive advantages over native species. Despite large numbers of species introductions followed by native species declines, relatively few studies provide experimental evidence documenting competitive interactions between native and invasive species (Lodge 1993; see reviews by Sakai et al. 2001; Levine et al. 2003).

Introductions of freshwater fish species provide opportunities to study competitive mechanisms occurring between native and nonnative species that may act to shape community structure. Many fish species are propagated in hatcheries and released into waterways to provide sport fisheries and as biological control agents. Aquarium releases and accidental transfer of fish species have also resulted in the establishment of nonnative fish populations. Over 500 nonnative fish species are reported in North America (Fuller et al. 1999), and several are implicated in the extinction of native fish populations. Although predation by nonnative species plays a role in the demise of some native fish species (Crowl et al. 1992), interference competition for limited resources such as food or habitat is probably common between ecologically similar native and nonnative fish species (Fausch 1988). Therefore, as populations of nonnative species become established within ecosystems, differences in interspecific competitive ability may play a large role in determining whether native fish species persist.

In western North America, the dramatic decline of cutthroat trout (*Oncorhynchus clarkii*) populations after the introduction of nonnative trout is of particular concern. Cutthroat trout have experienced drastic declines and extinctions because of habitat alteration and introduced fish species. Rainbow trout (*Oncorhynchus mykiss*) are considered a key reason for the loss of cutthroat trout populations (Young 1995), because cutthroat trout and rainbow trout are phylogenetically and ecologically similar. Cutthroat trout and rainbow trout are considered sister species and readily form fertile hybrid offspring when brought into sympatry (Krueger and May 1991; Behnke 1992). Of the 14 cutthroat trout subspecies endemic to North America, competition and hybridization with rainbow trout have been implicated in the extinction of one subspecies, the Alvord cutthroat trout (*Oncorhynchus clarkii alvordensis*, Bartley and Gall 1991) and designation as species of concern or protection under the US Endangered Species Act for several other subspecies (Allendorf and Leary 1988). Furthermore, the rapid loss of genetically pure cutthroat trout populations has been documented across many cutthroat trout subspecies after the introduction of rainbow trout (Jaeger et al. 2000; Hitt et al. 2003; Weigel et al. 2003), providing observational evidence for competitive exclusion. Although there is a pattern of cutthroat trout declines after rainbow trout introductions, there is little experimental evidence that determines whether cutthroat trout are competitively inferior to rainbow trout and cutthroat–rainbow hybrid trout.

In 2004, we began a series of studies to experimentally investigate potential competitive mechanisms related to swimming and foraging efficiency among native cutthroat trout, rainbow trout, and their hybrids. Salmonid fishes in streams primarily feed on drifting macroinvertebrates (Quinn 2005). Trout and salmon directly compete for feeding territories that provide access to high supplies of macroinvertebrates. Previous work by Seiler and Keeley (2007*a*, 2007*b*) indicates that cutthroat trout have lower sustained swimming ability than rainbow trout and hybrids as well as lower foraging success in contests against rainbow trout and hybrids. To assess the fitness implications of these differences, we conducted growth trials in experimental stream channels. We assayed the growth of cutthroat trout in allopatry and when in competition with similar size rainbow trout and first generation hybrid trout. Given our earlier studies and the pattern of declines and extinctions of native cutthroat trout populations after the introduction of rainbow trout (Jaeger et al. 2000; Hitt et al. 2003; Weigel et al. 2003), we hypothesized that cutthroat trout would exhibit lower growth rates when competing against rainbow trout or cutthroat–rainbow hybrids than when in allopatry.

Materials and methods

Collection and rearing of experimental animals

To control for potential environmental effects on the behavior and growth rate of fish in our study, gametes were collected from adult fish to create experimental cohorts and all offspring were reared in a common environment. On 4 April 2006, Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) were collected from a tributary stream of Henrys Lake, Island Park, Idaho, and rainbow trout from the Hayspur Hatchery, Hayspur, Idaho, USA. Eggs or sperm were removed from individual trout and transported to Idaho State University, where we created four genotypes: cutthroat trout, rainbow trout, and reciprocal crosses of first generation hybrids. Maternal cutthroat hybrid trout were created by fertilizing a clutch of cutthroat trout eggs with rainbow trout sperm, and maternal rainbow hybrid trout were created by fertilizing rainbow trout eggs with cutthroat trout sperm. To reduce the chance of using sperm from an infertile male, the sperm from two males were combined and used to fertilize a single clutch of eggs. This protocol was repeated three times per cross using unique fish each time (12 crosses total, three for each genotype). After fertilization, common genotypes were pooled into one incubation basket and suspended in a trough supplied with chilled (13 ± 1 °C) and aerated water.

Over the course of development, dead embryos were removed by hand several times a week. Although we did not quantitatively measure egg survival, cutthroat trout and maternal cutthroat hybrid trout had lower numbers of viable eggs 21 days after fertilization. On 1 May 2006, fertilized eggs were collected from the source populations to supplement the cutthroat trout and maternal cutthroat hybrid crosses. These embryos had been fertilized in a similar manner on 4 April 2006 and incubated at 13 °C (±1 °C) in Heath trays at the Henrys Lake Fish Hatchery. Approximately 3000 cutthroat trout and maternal cutthroat hybrid trout embryos were transported to Idaho State University and added to their respective incubation basket.

After hatching and development to the stage where all trout had absorbed their yolk sac and begun feeding, approximately 1200 fish from each genotype were transferred to separate compartments of a fiberglass rearing trough (Frigid-

Units Inc., Toledo, Ohio). Each compartment measured 48 cm long × 55 cm wide × 40 cm tall. A water chilling unit maintained temperature at 14 °C (±1 °C), and crosses were fed several times daily with standard hatchery feed (Biodry 1000, Bio-Oregon Inc., Warrenton, Oregon).

Experimental procedure

Given the close phylogenetic relationship of cutthroat trout and rainbow trout and the observation of native cutthroat trout population declines after rainbow trout introductions, we were interested in testing for the presence and strength of competition for cutthroat trout in allopatry versus competition with rainbow or hybrid genotypes. Therefore, we used a minimally substitutive experimental design (see Fausch 1998) to measure the competitive effect of rainbow trout, maternal rainbow hybrid trout, or maternal cutthroat hybrid trout on the growth of cutthroat trout. Although the hybrid genotypes are not technically considered separate species from cutthroat trout, we created them to model two possible hybrid genotypes that occur when rainbow trout and cutthroat trout co-occur and use the terms "interspecific" or "heterospecific" when referring to any treatment where cutthroat trout are paired with non-cutthroat trout cohorts.

To simulate an environment where the trout genotypes would compete for food resources as they would in wild populations, we built four identical stream channels by attaching a water pump to a fiberglass trough (240 cm × 55 cm × 25 cm). The pump drew water from two outlets on the downstream end and delivered it to a multi-outlet manifold on the upstream end of the channel. We maintained water velocity of each channel at 10 $cm \cdot s^{-1}$ (±2 $cm \cdot s^{-1}$) with a valve on the output of the pump and installed polyvinyl chloride (PVC) screens (3 mm diameter holes) located 20 and 15 cm from the downstream and upstream ends, respectively, to prevent fish from entering the pump and to create laminar flow along the length of the channel. We lined the bottom of each channel with a layer of stream gravel (mean size = 31.6 ± 9.67 mm standard deviation (SD), n = 100) and maintained water depth at 12 cm above the gravel using a standpipe drain. Owing to space and water supply limitations, two stream channels were positioned side by side, and the second set of channels was placed on an 88 cm tall stand above the first set. Chilled and aerated water (14 ± 1 °C) was gravity fed from a common reservoir to the top channels with overflow from the standpipes draining to the channel below it. Overflow from the bottom set of channels was collected in a common drain, filtered (mechanical, biological, ultraviolet (UV)), and pumped to the header reservoir. An input of fresh dechlorinated water (~3 $L \cdot m^{-1}$) to the header reservoir assured high water quality throughout the experiment. To provide a consistent photoperiod, a 100 W light was connected to a timer, mounted at 70 cm above the water surface of each channel, and set to a light regime of 13 h light:11 h dark.

On 12 July 2006, we began the experiment by establishing one replicate of fish in four experimental treatments: (*i*) cohorts of cutthroat trout in allopatry; (*ii*) cohorts of 1/2 cutthroat trout and 1/2 maternal cutthroat hybrid trout; (*iii*) cohorts of 1/2 cutthroat trout and 1/2 maternal rainbow hybrid trout, and (*iv*) cohorts of 1/2 cutthroat trout and 1/2 rainbow trout (Table 1). Given that relative size affects competitive ability of salmonid fishes (Abbott et al. 1985) as well as many other taxa (see table 9.1 in Archer 1988), fish were haphazardly sampled from each genotype so that fish within stream channels and between treatments were of similar size (mean ± 9 mm fork length). Individual trout were lightly anesthetized, measured for fork length (±0.5 mm) and wet mass (±0.0001 g) after blotting the fish with a paper towel, and tagged in the snout with a sequential coded wire tag (sCWT, Northwest Marine Technology Inc., Shaw Island, Washington, USA). Juvenile cutthroat trout, rainbow trout, and cutthroat–rainbow trout hybrids have very similar phenotypes; hence, sCWTs allowed us to identify individual trout with minimal stress relative to other tagging methods (i.e., latex injection, fin clips). To equalize the density of trout within troughs while accounting for small size differences between individual trout, each channel was stocked according to the percent habitat saturation model (PHS) of Grant and Kramer (1990). PHS was developed to estimate the maximum density of salmonid fishes in stream environments based on the territory size of individual fish (Grant and Kramer 1990); a PHS of 100% represents a stream where all potential habitat is filled with foraging territories. For cutthroat trout in the allopatry treatment, we measured and tagged enough fish to set the PHS of the stream channel between 108% and 116% (Table 1). For the other treatments, an equal number of cutthroat trout and the competitor genotype was added until reaching PHS between 105% and 117% (Table 1). Trout for all experimental cohorts were allowed to recover from anesthesia in mesh baskets suspended in the rearing trough until the trials began. On the following day, each cohort was introduced to a randomly chosen stream channel. An automated belt feeder (Aquatic Ecosystems, Apopka, Florida, USA) was attached to the upstream end of each channel, and we filled each stream channel's feeder with a ration of food beginning at 0730 each day. We spread hatchery feed across the entire belt feeder to deliver food over a 12 h period and simulate an encounter rate of prey items that fish experience in natural streams (Keeley and Grant 1995). The daily food ration for each channel was based on the size of each fish in the trough at the time of stocking and equal to 100% of the maximum ration predicted by Marschall and Crowder's (1995) model for juvenile salmonid fishes. Expressed as the percentage of body mass for all trout in each stream channel, the daily food ration equaled 4.3% to 4.9% of the wet mass of trout at the start of the experiment. After 7 days, we adjusted the food ration to 75% of the maximum predicted ration (3.2% to 3.7% according to total starting wet weight of trout in each channel) to intensify competition for food resources.

Twenty days after adding cohorts to the channels, all trout were collected, euthanized with an overdose of anesthesia, and individually measured for length (±0.5 mm) and wet mass (±0.0001 g) after blotting with a paper towel. The sCWTs from each fish were retrieved to identify individual fish and allow calculation of change in mass. Between 12 July 2006 and 3 October 2006, we conducted four replicates of the four experimental treatments using new fish from the rearing cohorts for each replicate. Although we did not expect the position of treatment groups within stream channels

Table 1. Size distribution of trout and percent habitat saturation (PHS) within stream channels for each treatment.

Treatment	Replicate	No. of YCT	No. of heterospecific trout	Mean fork length (cm ± 1 SE) YCT	Heterospecific	PHS
YCT in allopatry	1	26	—	3.92 (0.03)	—	108
	2	24	—	4.03 (0.03)	—	112
	3	24	—	4.05 (0.04)	—	112
	4	23	—	4.16 (0.03)	—	115
YCT vs. MCH	1	13	13	3.76 (0.04)	3.75 (0.05)	104
	2	12	12	3.95 (0.05)	3.92 (0.04)	109
	3	12	12	3.95 (0.05)	3.95 (0.04)	110
	4	13	13	3.91 (0.03)	3.96 (0.04)	116
YCT vs. MRH	1	12	12	3.99 (0.04)	3.98 (0.05)	113
	2	11	11	4.11 (0.05)	4.11 (0.04)	112
	3	11	11	4.17 (0.09)	4.18 (0.08)	117
	4	10	10	4.24 (0.08)	4.28 (0.08)	112
YCT vs. RBT	1	12	12	3.94 (0.07)	3.93 (0.06)	109
	2	12	12	4.01 (0.06)	4.00 (0.05)	114
	3	12	12	4.00 (0.05)	4.00 (0.05)	114
	4	11	11	4.16 (0.04)	4.17 (0.04)	116

Note: YCT, Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*); MCH, maternal cutthroat hybrid trout; MRH, maternal rainbow hybrid trout; RBT, rainbow trout (*Oncorhynchus mykiss*); SE, standard error.

to influence growth of trout between treatments, treatment groups were rotated to each channel over the remaining three replicates.

Statistical analyses

As a measure of the presence and strength of competition, we compared the growth rate of cutthroat trout cohorts among the four treatment levels. By making experimental observations on only cutthroat trout growth, we made an independent observation of competitive interactions between cutthroat trout in allopatry and the cutthroat trout in the other three treatments. Over the course of the experiment, there was no mortality of trout stocked into stream channels; however, some cutthroat trout could not be identified at the end of the experiment because of sCWT loss (13 out of 238 cutthroat trout) and were not included in the analysis of growth. Although our method for stocking each stream channel created similar size distributions within each channel, we could not maintain identical sizes within our rearing cohorts over the course of the experiment. To account for these size differences, we analyzed individual growth as the daily instantaneous growth rate (g) using the following formula:

$$G = \ln(W_t) - \ln(W_i)/(t_2 - t_1)$$

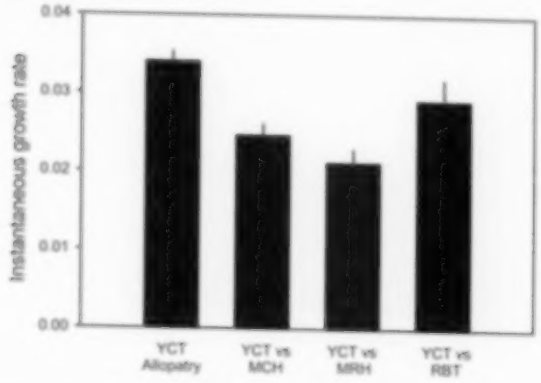
where G is the daily growth rate, W_i is the initial wet mass (g), W_t is the final wet mass (g), and $(t_2 - t_1)$ was equal to 20 days. We compared the growth rate of cutthroat trout between treatments using an analysis of variance (ANOVA) with the mean growth of the entire cohort of cutthroat trout within a stream channel as the unit of observation (16 observations) and a blocking term to account for differences in starting time of the replicates. To determine which treatment levels differed from each other, we used planned a priori contrasts to compare the growth of cutthroat trout cohorts in allopatry with the growth of cutthroat trout cohorts in each heterospecific treatment as well as against the growth of cutthroat trout cohorts in all heterospecific treatments combined.

Although the main goal of this study was to test for the presence and strength of competition on cutthroat trout cohorts, we conducted a secondary analysis to investigate the growth rate of individual cutthroat trout in different competitive environments. Within cohorts, the size distribution of cutthroat trout and heterospecific genotypes may influence which cutthroat trout can best compete for profitable foraging locations. If relative size of individual cutthroat trout determines success at capturing food within a cohort of competitors, we expected cutthroat trout that were smaller than the average size trout in a stream channel to experience lower growth rate and larger cutthroat trout to experience higher growth rate. Therefore, we calculated a covariate of relative size by dividing the initial mass of each cutthroat trout by the mean mass of all trout in its stream channel. We then used an analysis of covariance (ANCOVA) to compare the initial relative mass value and growth rate for individual cutthroat trout within each treatment. All statistical tests were performed in SAS 9.1 (SAS Institute Inc. 2003), with significance evaluated at $\alpha = 0.05$.

Results

Over the 20-day trials, we detected large differences in the growth of cutthroat trout cohorts across treatment levels. Cutthroat trout growth rate was different among the four treatments (ANOVA, $F_{[3,12]} = 9.53$, $P = 0.0037$), with the highest growth rate occurring in the allopatric cutthroat trout treatment (Fig. 1). The blocking factor did not account for a significant proportion of the variability in growth rate (ANOVA, $F_{[3,15]} = 1.35$, $P = 0.32$), indicating that timing and initial size of trout cohorts over the course of the experi-

Fig. 1. Growth rate for cutthroat trout cohorts in allopatry and in sympatry with hybrid trout or rainbow trout. Bars represent the mean growth rate (+1 standard error, SE) of cutthroat trout cohorts for four replicates of each treatment. YCT, Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*); MCH, maternal cutthroat hybrid trout; MRH, maternal rainbow hybrid trout; RBT, rainbow trout (*Oncorhynchus mykiss*).



ment was unimportant. When compared according to individual treatments, cutthroat trout growth was lower within the maternal cutthroat hybrid and maternal rainbow hybrid treatments than cutthroat trout growth in allopatry (ANOVA, $F_{[1,7]} = 13.60$ and 25.26, $P = 0.005$ and <0.001, respectively). The growth rate of cutthroat trout when rainbow trout were present was lower but not significantly different from cutthroat trout growth in allopatry (ANOVA, $F_{[1,7]} = 3.66$, $P = 0.088$). When compared against all heterospecific treatments combined, cutthroat trout growth in allopatry was higher than cutthroat trout growth in sympatry (ANOVA, $F_{[1,15]} = 18.82$, $P = 0.0019$).

For the analysis of individual cutthroat trout relative size and growth, we detected an effect of the initial relative mass on growth rate ($F_{[1,217]} = 22.3$, $P < 0.0001$), indicating cutthroat trout that were smaller than the average trout within a stream channel grew less than the average cutthroat trout. However, we also detected a treatment effect for individual cutthroat trout growth (ANCOVA, $F_{[3,217]} = 6.85$, $P = 0.0002$), providing further evidence that differences in growth rate exist for cutthroat trout in the presence of heterospecific genotypes. Similarly, a significant interaction between initial relative size and treatment (ANCOVA, $F_{[3,217]} = 5.75$, $P = 0.0008$) indicates that the slope of the regression lines for relative size and growth rate among treatments were different; there is no relationship between initial relative size and growth rate for cutthroat trout in allopatry, while these same relationships for cutthroat trout in channels with maternal cutthroat trout hybrids, maternal rainbow trout hybrids, and rainbow trout have positive slopes significantly different from zero (Fig. 2).

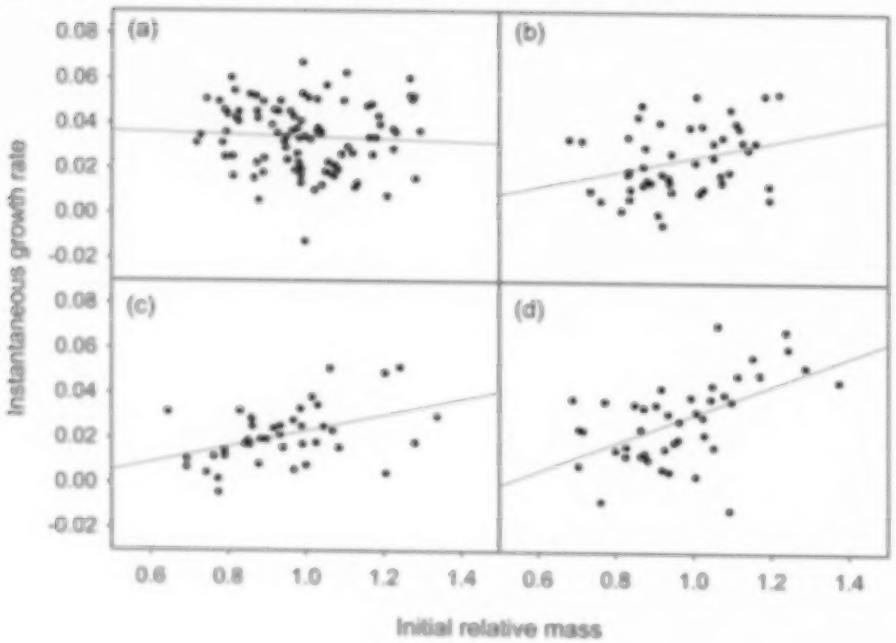
Discussion

Several recent studies have documented the spread of introduced rainbow trout and cutthroat–rainbow hybrids within cutthroat trout populations (c.f., Hitt et al. 2003; Rubidge and Taylor 2005; Gunnell et al. 2008), bringing attention to the drastic decline of native cutthroat trout and reinforcing ideas from earlier studies suggesting that rainbow trout genotypes out-compete native cutthroat trout. Our previous studies (Seiler and Keeley 2007*a*, 2007*b*) illustrate some of the potential competitive differences among cutthroat trout, rainbow trout, and their first generation hybrids, with cutthroat trout exhibiting lower sustained swimming ability and reduced foraging success when competing against rainbow trout or hybrid genotypes. This study suggests that swimming and foraging differences may have implications for population-level processes; cutthroat trout cohorts in the presence of either hybrid genotype or all heterospecific treatments combined exhibit a lower growth rate than cutthroat trout in allopatry.

Although the growth rate of cutthroat trout cohorts in sympatry with rainbow trout was not significantly lower than growth of cutthroat trout cohorts in allopatry, we believe that this result warrants further research. Given the low feeding rate of cutthroat trout in foraging contests with rainbow trout (Seiler and Keeley 2007*b*), we expected cutthroat trout growth rate to be lowest in channels with rainbow trout. A possible explanation for higher than expected growth from these cohorts could be that aggression among rainbow trout was higher than aggression among maternal cutthroat trout hybrids and maternal rainbow trout hybrids as suggested by Seiler and Keeley (2007*b*). If rainbow trout spent a larger proportion of time than either hybrid genotype fighting conspecific genotypes for foraging positions in the stream channels, a greater proportion of food items may have been delivered to cutthroat trout in downstream foraging positions. If we had been able to run our experimental replicates for longer time intervals, we would expect rainbow trout feeding hierarchies to become better established with lower intraspecific aggression; the majority of cutthroat trout, defending downstream positions in the stream channel, would then exhibit a reduction in growth rate.

By collecting data from individual trout within treatments, our study also provides an opportunity to better understand how nonnative species may competitively supplant native species. In salmonid fishes, even small differences in relative size and prior residency can influence the success of acquiring and maintaining a profitable foraging location (Abbott et al. 1985; Cutts et al. 1999; Metcalfe et al. 2003). When comparing growth of individual cutthroat trout within heterospecific competition treatments, we found a positive relationship between initial relative mass and growth rate, indicating that relative size of individual cutthroat trout influenced their ability to acquire food and grow. Cutthroat trout that were smaller than the average trout within these channels fared poorly, while those larger than the average grew as well or better than cutthroat trout in allopatry. These relationships suggest that over the size range within our stream channels, the intensity of competition between cutthroat trout and heterospecific genotypes was only overcome by the largest cutthroat trout. Additionally, in the cohort analysis, we did not detect a difference in growth rate between cutthroat trout in allopatry and cutthroat trout in channels with rainbow trout, despite individual cutthroat trout in channels with rainbow trout exhibiting the same strong relationships between initial mass and growth rate existing for cutthroat trout in channels with hybrid genotypes.

Fig. 2. Relationships between the growth rate and initial relative mass of individual Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) among the four treatments. (*a*) Cutthroat trout in allopatry. Equation of the line: instantaneous growth rate = –0.005 × initial relative mass + 0.039 (r^2 = 0.003, n = 94, P = 0.64); (*b*) cutthroat trout in sympatry with maternal cutthroat hybrid trout. Equation of the line: instantaneous growth rate = 0.034 × initial relative mass – 0.008 (r^2 = 0.097, n = 47 P = 0.033); (*c*) cutthroat trout in sympatry with maternal rainbow hybrid trout. Equation of the line: instantaneous growth rate = 0.035 × initial relative mass – 0.012 (r^2 = 0.20, n = 41, P = 0.004); (*d*) cutthroat trout in sympatry with rainbow trout (*Oncorhynchus mykiss*). Equation of the line: instantaneous growth rate = 0.065 × initial relative mass – 0.033 (r^2 = 0.31, n = 43, P < 0.0001).



Similar to findings by Forseth et al. (2003) and Blanchet et al. (2007), our analyses illustrate that individual-level effects of interspecific competition may be concealed at the population level, and thus we recommend that future studies assessing potential competitive influences between native and nonnative species use multiple scales of inference.

Our study is particularly important for understanding the implications of competitive interactions between native and introduced salmonid fishes because the ability of juvenile salmonid fishes to acquire food and grow during a short window of time after hatching is thought to be critical for survival (Elliott 1994). Juvenile trout and salmon that cannot acquire feeding territories in streams either die or are forced to emigrate in search of profitable feeding positions (Elliott 1994; Keeley 2001). Therefore, any competitive disadvantage that young-of-the-year cutthroat trout experience through competition with rainbow trout and cutthroat–rainbow hybrids is likely to have important consequences for the persistence of native cutthroat trout populations. Unlike Keeley (2001), we did not provide a means for competitively inferior fish to emigrate from the streams channels. Given the disparity in mass of cutthroat trout and heterospecific competitors at the end of our experiment, we would expect mortalities of the smaller cutthroat trout to occur if we had extended the experimental treatment beyond 20 days.

In our study, we aimed to test for the effect of competition on cutthroat trout in the presence of different competitors when trout were of similar size; however, the discrepancy in size and prior residency for rainbow trout, hybrid trout, and cutthroat trout may have important interactions in wild populations. In the South Fork of the Snake River of Idaho and Wyoming, USA, a proportion of wild rainbow trout and cutthroat–rainbow hybrids spawn earlier than the native Yellowstone cutthroat trout (Henderson et al. 2000; De Rito 2004). Young-of-the-year rainbow trout and hybrid trout may therefore emerge earlier and possess a size and prior residency advantage over later emerging Yellowstone cutthroat trout. Validation of the timing of emergence and relative size of young-of-the-year cutthroat trout, rainbow trout, and hybrids upon emergence is possible but would require intensive sampling and genetic analyses because phenotypic differences between these crosses are difficult to identify in the field. However, a long-term survey of trout populations in the South Fork of the Snake River provides evidence consistent with this hypothesis. Despite a reduction in the number of rainbow trout stocked into the South Fork of the Snake River, the proportion of juvenile Yellowstone cutthroat trout has declined, while rainbow trout and hybrids have increased (Schrader and Fredericks 2006). Furthermore, Yellowstone cutthroat trout are approximately 20% smaller in length than rainbow trout and hybrids after their first summer of growth (Schrader and Fredericks 2006), suggesting that timing of emergence, ini-

tial size differences, or prior residency result in large discrepancies in size among these genotypes. These studies in natural populations suggest that the growth rate differences we observed will only be magnified when rainbow trout and cutthroat–rainbow trout hybrids begin with size and residency advantages.

Although our study represents some of the first experimental evidence to indicate that cutthroat trout populations are at a competitive disadvantage to hybrid trout, additional factors thought to influence competitive interactions among stream-dwelling fishes are needed to fully understand how introduced salmonids may interact with native species. For example, we specifically chose hatchery rainbow trout to mimic the common practice of releasing hatchery fish into native trout populations. However, past studies have found that populations of hatchery salmonids tend to have higher levels of aggression than wild populations (reviewed by Weber and Fausch 2003), but genetically-based differences in aggression among populations and the resulting population-level implications have rarely been evaluated. Similarly, the common pattern of cutthroat trout occupying small headwater streams has led some to propose that cutthroat trout are competitively excluded in larger streams with warmer water temperatures (Griffith 1988; Bozek and Hubert 1992). However, surveys of rainbow trout and cutthroat–rainbow hybrid trout within native cutthroat trout populations in the South Fork of the Snake River, Idaho, did not find a strong association between the distribution of rainbow trout and hybridized trout and summer stream temperature or other habitat characteristics (Seiler 2007). Despite the potential importance of these patterns, few studies have demonstrated how differences in competitive ability may translate into population consequences for cutthroat trout, but our study provides a baseline of how cutthroat trout growth rates are affected by competition from rainbow trout and cutthroat–rainbow hybrids.

The evolutionary history of cutthroat trout and rainbow trout may provide additional insight into why rainbow trout and cutthroat–rainbow hybrids are successful when introduced into native cutthroat trout streams. Unlike many terrestrial organisms, fish populations are often isolated from adjacent conspecific and heterospecific populations because of discrete barriers to movement. Yellowstone cutthroat trout, as well as most other cutthroat trout subspecies, evolved in isolation from any other trout species (Gresswell 1995). Because they did not experience competition from other similar salmonid species, cutthroat trout may have evolved as generalists (Griffith 1988). A long history without interspecific competitors may make Yellowstone cutthroat trout particularly susceptible to competition from ecologically similar introduced salmonids (Griffith 1988; Gresswell 1995). In contrast, rainbow trout evolved with a suite of ecologically similar species including Pacific salmon (*Oncorhynchus* sp.) and char (*Salvelinus* sp.; McPhail and Lindsey 1986; Quinn 2005). Hence, repeated stocking of rainbow trout into inland river ecosystems (high propagule pressure; Kolar and Lodge 2001) with relatively simple fish faunas where many cutthroat trout subspecies evolved may promote competitive exclusion of native cutthroat trout.

Several studies in ecosystems where hybridization occurs between native and introduced species illustrate patterns similar to hybridization between cutthroat trout and rainbow trout. Hybrids between a native and introduced crayfish (*Orconectes* sp.) are dominant over both parental species for limited food resources (Perry et al. 2001), and hybrids between Pecos pupfish (*Cyprinodon pecosensis*) and introduced sheepshead minnow (*Cyprinodon variegatus*) have greater swimming stamina than the native pupfish (Rosenfield et al. 2004). These studies provide examples of hybrid heterosis (Arnold 1997), and accordingly, the lower growth rate of Yellowstone cutthroat trout in treatments with maternal cutthroat trout hybrids and maternal rainbow trout hybrids, coupled with the findings from Seiler and Keeley (2007*a*, 2007*b*), provides evidence that juvenile cutthroat–rainbow trout hybrids in the wild probably have advantages over native cutthroat trout. However, hybridization can lead to genomic extinction of native species even when the fitness of hybrids is less than that of parental species by propagating nonnative genes into native populations over time (Huxel 1999; Epifanio and Philipp 2001). Therefore, any fitness advantages that hybrids experience will only serve to hasten the loss of genetically pure native populations. Future studies of ecosystems where hybridization occurs should aim to measure traits correlated with fitness (i.e., growth, survivorship, spawning success) of native species, introduced species, and their hybrids.

Ecologists are making progress at identifying traits common to invasive taxa and ecosystems prone to invasion (Elton 1958; Rejmanek and Richardson 1996; see review by Kolar and Lodge 2001). A stronger quantitative understanding of individual invasions has been called for to achieve the ultimate goal of invasive species ecology: the prediction and prevention of future species invasions (Kolar and Lodge 2001). Similar to studies by Petren and Case (1996) and Holway (1999), our study provides evidence that competition can play an important role in the spread of nonnative species. Additionally, hybridization between cutthroat trout and rainbow trout and the processes described above present special opportunities to study a type of ecological invasion considered to have strong impacts on native species (Rhymer and Simberloff 1996). Further studies that elucidate mechanisms of competition acting between native species and introduced species when hybridization occurs, such as Perry et al. (2001) and Byers and Goldwasser (2001), will add valuable information to the study of invasive species ecology and community ecology in general.

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Ages and growth rates of some deep-sea gorgonian and antipatharian corals of Newfoundland and Labrador

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Abstract: Using bomb-^{14}C dating and growth ring counting methods, we calculate life spans and growth rates of six species of deep-sea corals collected at depths of between 400 and 900 m from the continental slope of Newfoundland and Labrador. Bamboo corals (*Acanella arbuscula*, *Keratoisis ornata*) and antipatharians (*Stauropathes arctica*) secrete concentric growth rings in their axial skeletons, which were proven to form annually for *K. ornata* and *S. arctica*. These species had the lowest radial growth rates of 33 ± 11 to 75 ± 11 μm·year^{-1}. *Primnoa resedaeformis* and *Paramuricea* spp. had higher radial growth rates of 83 ± 6 to 215 ± 37 μm·year^{-1}. *Paragorgia arborea* had the highest radial growth rate of 830 ± 120 μm·year^{-1}. Axial growth rates ranged from 0.56 ± 0.05 cm·year^{-1} for a specimen of *Paramuricea* spp. to 2.61 ± 0.45 cm·year^{-1} for a specimen of *Primnoa resedaeformis*. Life spans ranged from 18 ± 4 years for a live-collected *P. resedaeformis* to 200 ± 30 years for a subfossil specimen of *K. ornata*. Because all of the corals were from heavily fished areas, it is likely that age distributions are biased towards smaller and younger colonies. Recovery of deep-sea corals from fishing-induced damage will take decades to centuries.

Résumé : L'utilisation de méthodes de datation basées sur le ^{14}C des essais nucléaires et le dénombrement des anneaux de croissance nous a permis de déterminer la durée de vie et les taux de croissance de six espèces de coraux marins d'eau profonde récoltées sur la pente continentale de Terre-Neuve et du Labrador, à des profondeurs de 400 m à 900 m. Les coraux bambous (*Acanella arbuscula*, *Keratoisis ornata*) et antipathariens (*Stauropathes arctica*) sécrètent des anneaux de croissance concentriques sur leur squelette axial et il est démontré que chez *K. ornata* et *S. arctica* ces anneaux se forment annuellement. Ces espèces possèdent les taux de croissance radiale les plus bas, de 33 ± 11 μm·an^{-1} à 75 ± 11 μm·an^{-1}. *Primnoa resedaeformis* et *Paramuricea* spp. ont des taux de croissance radiale plus élevés, 83 ± 6 μm·an^{-1} à 215 ± 37 μm·an^{-1}. *Paragorgia arborea* présente le taux de croissance radiale le plus élevé, 830 ± 120 μm·an^{-1}. Les taux de croissance axiale varient de 0,56 ± 0,05 cm·an^{-1} chez un spécimen de *Paramuricea* spp. à 2,61 ± 0,45 cm·an^{-1} chez un spécimen de *P. resedaeformis*. Les durées de vie vont de 18 ± 4 années chez un *P. resedaeformis* récolté vivant à 200 ± 30 ans pour un spécimen subfossile de *K. ornata*. Puisque tous les coraux proviennent de régions soumises à une pêche intensive, il est vraisemblable que les distributions d'âge favorisent les colonies plus petites et plus jeunes. La récupération des coraux marins d'eau profonde des dommages infligés par la pêche prendra des décennies et même des siècles.

[Traduit par la Rédaction]

Introduction

In recent years there has been increasing interest in the ecology and biology of deep-sea or cold-water coral ecosystems (Freiwald and Roberts 2005; Roberts et al. 2006). Off eastern Canada, deep-sea coral ecosystems comprise gorgonian and antipatharian "coral forests" located predominantly along the upper continental slope at depths of between 200 and 1000 m (Gass and Willison 2005; Mortensen et al. 2006; Wareham and Edinger 2007). These structurally complex ecosystems support a diversity of associated invertebrates and fish (Krieger and Wing 2002; Stone 2006; Edinger et al. 2007*a*). Deep-sea corals are highly vulnerable to the impacts of bottom trawling (Koslow et al. 2001; Hall-Spencer et al. 2002; Edinger et al. 2007*b*). Unfortunately, widespread industrialization of fishing fleets off eastern Canada and in other regions predates much of the recent scientific interest in deep-sea corals. As a result, trawling impacts to coral habitats may have gone on virtually unnoticed for decades (Haedrich et al. 2001; Kulka and Pitcher 2002).

Information on growth rates and life spans of deep-sea corals is important for their conservation and management. These parameters are vital for assessing the vulnerability of these organisms to natural and anthropogenic disturbances and the time scale of their recovery. One of the dominant gorgonian species off eastern Canada, *Primnoa resedaeformis*, is known to live for at least 700 years (Sherwood et al.

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2006). In this paper, we measure growth rates and life spans of the gorgonians *Acanella arbuscula*, *Keratoisis ornata*, *Paramuricea* spp., and *Paragorgia arborea* and antipatharian *Stauropathes arctica* collected off Newfoundland and Labrador, using the bomb-radiocarbon dating method. We also compare radial and linear growth rates in a suite of growth ring dated colonies of *P. resedaeformis* collected during a single tow in the northern Labrador Sea with growth rates from the Northeast Channel, southwest of Nova Scotia.

Oceanic uptake of bomb radiocarbon produced by atmospheric nuclear weapons testing in the late 1950s and early 1960s provides a time-varying tracer. Based on direct measurements of dissolved inorganic carbon (DIC; Nydal et al. 1998) and indirect measurements of ^{14}C in known-age proxies (Druffel 1980; Weidman and Jones 1993), the initial increase in bomb ^{14}C in surface waters of the North Atlantic occurred around 1957. This provides a unique time marker that may be used to establish or validate skeletal chronology (Kerr et al. 2005; Roark et al. 2006). If a reference bomb-^{14}C chronology is known for a particular water mass, then that itself may be used for age calibration of bomb-^{14}C data (Campana 1997; Roark et al. 2005; Sherwood et al. 2005). Potential carbon sources to deep-sea corals include particulate organic carbon (POC), sedimentary organic carbon (SOC), dissolved organic carbon (DOC), and DIC (Roark et al. 2006). Below the mixed layer of the ocean, recently exported POC is generally the only carbon source "tagged" with bomb ^{14}C; other carbon reservoirs contain typically prebomb levels of $\Delta^{14}C$ (<0‰; Druffel and Williams 1990; Druffel et al. 1992; Bauer et al. 2001). Because the organic fractions of deep-sea corals contain bomb ^{14}C, recently exported POC is thought to be their main carbon source (Griffin and Druffel 1989; Roark et al. 2005; Sherwood et al. 2005). Therefore, bomb-^{14}C measurements may be used to date deep-sea corals, assuming that there is no phase-lag or signal attenuation in the bomb-^{14}C signal in recently exported POC with depth in the water column.

Materials and methods

Since 2002, coral specimens caught opportunistically as trawl bycatch during stock assessment surveys and fisheries operations have been collected and stored by Fisheries and Oceans Canada. The sampling program and the deep-sea coral fauna of the region are described more completely in Wareham and Edinger (2007) and Edinger et al. (2007*a*). From this collection, colonies of *A. arbuscula* (Johnson, 1862), *S. arctica* (Lütken, 1871), *K. ornata* (Verrill, 1878), *P. arborea* (Linnaeus, 1758), *P. resedaeformis* (Gunnerus, 1763), and *Paramuricea grandis* (Verrill, 1884) and (or) *Paramuricea placomus* (Linnaeus, 1758) were selected. As much as possible, representative colonies of each species were selected; however, it was necessary in some cases to select larger colonies to maximize sample mass for ^{14}C analyses. Additional samples were collected using the remotely operated vehicle *ROPOS* during an expedition to the Southwest Grand Banks in July 2007. Complete sample details are provided in Table 1.

For radiocarbon analysis, approximately 0.5 cm thick cross sections were cut from the bases of colonies with an Isomet™ low-speed saw or a hand saw. The sections were ground and polished using silicon carbide films, ultrasonically cleaned in deionized water, and then photographed with a Nikon Coolpix™ 900 digital camera attached to a Nikon SZ 1000 binocular microscope. Incandescent or ultraviolet light was used to image growth rings, and resulting images were used to guide sampling. Samples were isolated in one of three ways. Isidid gorgonian corals (*A. arbuscula*, *K. ornata*) were prepared by sectioning the organic nodes from calcitic internodes and dissolving the sections in 5% HCl to remove any remaining carbonate. Individual growth rings were then teased apart with scalpel and tweezers (Sherwood et al. 2005). For the spongy skeletons of *P. arborea* and *Paramuricea* spp., a wedge was cut from each section and subsampled along its radius with a stainless-steel scalpel. Finally, *S. arctica* colonies were sectioned in half longitudinally along the skeletal axis. One-half of the longitudinal section was affixed to a glass slide with thermoplastic glue and sampled with a manual micromill. Each sample measured approximately 10 mm long × 0.5 mm wide × 1.5 mm deep, giving an approximate sample mass of 2 mg.

Samples were transferred to 5 mL polyethylene vials, topped with 5% HCl, and left for 24 h to ensure that all of the calcite dissolved. Afterwards, samples were triple-rinsed in deionized water and dried at 70 °C overnight. Decalcification was not done for *S. arctica* as it does not contain any carbonate. Samples were combusted in individual quartz tubes and reduced to graphite in the presence of iron catalyst. Radiocarbon measurements were performed on graphite targets at the Centre for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. Results include a background and $\delta^{13}C$ correction and are reported as $\Delta^{14}C \pm 1\sigma$ error according to Stuiver and Polach (1977). Measurement of $\delta^{13}C$ was performed at Memorial University on a Finnigan™ MAT252 isotope ratio mass spectrometer following standard procedures, and values ranged from −20‰ to −17‰.

Calibration of bomb-^{14}C data

Coral $\Delta^{14}C$ data were calibrated using bomb-^{14}C reference chronology (Fig. 1*a*), based on three independently dated colonies of *P. resedaeformis* collected near the eastern entrance to Hudson Strait (samples 1525-1, 1525-3, 1525-10; 60°30′N, 61°24′W) and one colony of *K. ornata* from the southwest Grand Banks (sample 1343; 44°7′N, 52°56′W; Sherwood et al. 2008). The *P. resedaeformis* colonies were dated by counting the annual growth rings viewed in cross sections. Previous work has proven that growth rings in this species form with annual periodicity in both Northeast Pacific (Andrews et al. 2002) and Northwest Atlantic environments (Sherwood et al. 2005). Each colony was dated by three amateur counters, as described in Sherwood et al. (2005). Calendar ages were averaged over the three trials, and resulting mean ages were paired with the individual rings isolated for ^{14}C analysis. The colony of *K. ornata* was dated by conventional ^{210}Pb dating of a calcite internode sectioned from the base of the colony. The ^{210}Pb data were used to calculate the growth rate and the age of the section from the margin to the core by fitting the data with a decay curve with the ^{210}Pb decay constant (0.03067·year^{-1}). The adjacent gorgonin node, sampled for

Table 1. Collection details, ages, and growth rates (GR) of antipatharian and gorgonian (Order Alcyonacea) corals collected off Newfoundland and Labrador.

Order/Family/Species	Sample identification	Latitude (N)	Longitude (W)	Depth (m)	Year of collection	Dating method	Age (years)	Radial GR ($\mu m \cdot year^{-1}$)	Axial GR ($cm \cdot year^{-1}$)
Antipatharia									
Schizopathidae									
Stauropathes arctica	465	44°34.02′	53°46.02′	876	2005	Bomb ^{14}C	55±8	66±11	1.36±0.20
Stauropathes arctica	2440	44°57.45′	55°1.19′	812	2007	Bomb ^{14}C	82±31	33±11	1.22±0.46
Alcyonacea									
Isididae									
Acanella arbuscula	1591	65°7.86′	58°27.12′	526	2006	Bomb ^{14}C[c]	<100	>20	>0.30
						Growth rings[d]	30	70	1.00
Keratoisis ornata	2452	44°49.96′	54°28.13′	601	2007	Bomb ^{14}C	94±7	74±6	0.93±0.08
Keratoisis ornata	1449[a]	61°36.00′	60°22.98′	1193	2007	^{14}C	200±30	75±11	n/a
Keratoisis ornata	1343[b]	44°7.98′	52°55.98′	713	2006	^{210}Pb	138±23	53±9	n/a
Plexauridae									
Paramuricea spp.	1348	44°13.02′	53°0.00′	814	2006	Bomb ^{14}C	103±14	92±18	0.58±0.08
Paramuricea spp.	1886	55°13.14′	55°10.98′	850	2006	Bomb ^{14}C	71±6	205±20	0.56±0.05
Paragorgiidae									
Paragorgia arborea	1523	60°29.88′	61°23.70′	414	2006	Bomb ^{14}C	80±11	830±120	1.62±0.22
Primnoidae									
Primnoa resedaeformis	1525-1[b]	60°29.88′	61°23.70′	414	2006	Growth rings[e]	50±3	n/a	n/a
Primnoa resedaeformis	1525-3[b]	60°29.88′	61°23.70′	414	2006	Growth rings[e]	100±9	90±8	1.00±0.09
Primnoa resedaeformis	1525-4	60°29.88′	61°23.70′	414	2006	Growth rings[f]	72±4	146±8	n/a
Primnoa resedaeformis	1525-5	60°29.88′	61°23.70′	414	2006	Growth rings[f]	31±4	165±21	1.71±0.22
Primnoa resedaeformis	1525-6	60°29.88′	61°23.70′	414	2006	Growth rings[f]	23±4	215±37	2.61±0.45
Primnoa resedaeformis	1525-7	60°29.88′	61°23.70′	414	2006	Growth rings[f]	24±4	188±31	2.50±0.42
Primnoa resedaeformis	1525-8	60°29.88′	61°23.70′	414	2006	Growth rings[f]	24±4	167±28	2.29±0.38
Primnoa resedaeformis	1525-10[b]	60°29.88′	61°23.70′	414	2006	Growth rings[e]	44±3	125±9	1.86±0.13
Primnoa resedaeformis	1526-1	60°10.56′	61°47.40′	257	2006	Growth rings[f]	50±4	130±10	n/a
Primnoa resedaeformis	1526-2	60°10.56′	61°47.40′	257	2006	Growth rings[f]	57±4	105±7	n/a
Primnoa resedaeformis	1534	61°29.28′	61°49.14′	608	2006	Growth rings[f]	52±4	83±6	n/a
Primnoa resedaeformis	1536-1	61°12.12′	61°22.68′	585	2006	Growth rings[f]	18±4	194±43	1.94±0.43
Primnoa resedaeformis	1536-2	61°12.12′	61°22.68′	585	2006	Growth rings[f]	20±4	170±34	1.80±0.36
Primnoa resedaeformis	1539	60°19.38′	62°8.94′	285	2006	Growth rings[f]	18±4	181±40	n/a
Primnoa resedaeformis	1567	62°9.66′	61°32.22′	456	2006	Growth rings[f]	62±4	121±8	n/a
Primnoa resedaeformis	2370-1	61°21.00′	60°40.98′	640	2006	Growth rings[f]	42±4	194±18	1.90±0.18

Note: Additional samples not dated by the bomb-^{14}C method are included for comparison. n/a, not available.

[a]Subfossil specimen (Sherwood et al., unpublished data).

[b]Specimens included in reference chronology (Sherwood et al., unpublished data).

[c]Too few bomb-^{14}C data points to constrain absolute age and growth rate.

[d]Assuming growth rings are formed annually.

[e]Growth ring counting followed procedure in Sherwood et al. (2005) using three counters. Error reported as ±1 SD.

[f]Growth rings counted by one counter. Age error estimated as ±4 years following Sherwood et al. (2005).

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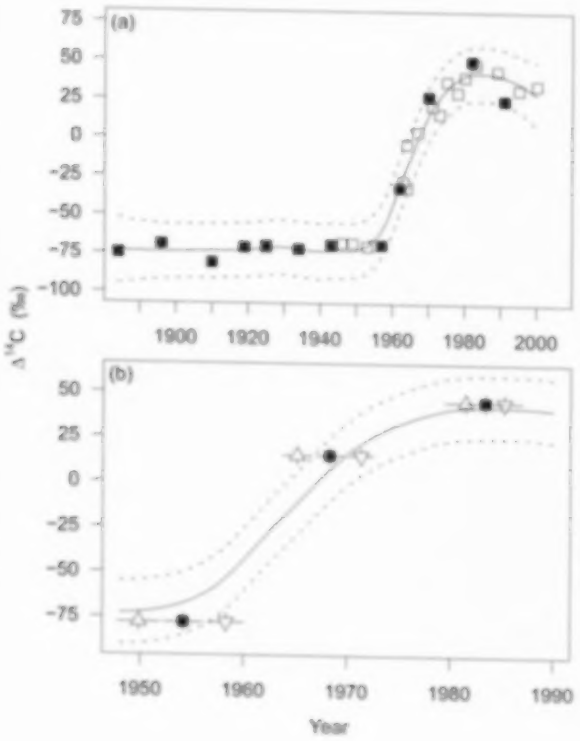
Fig. 1. (*a*) Bomb-^{14}C reference chronology for the offshore branch of the Labrador Current off Newfoundland and Labrador, derived from growth ring dated colonies of *Primnoa resedaeformis* from the Hudson Strait (□) and a ^{210}Pb-dated colony of *Keratoisis ornata* from the Grand Banks (■; Sherwood et al. 2008). Data have been fitted with a Loess curve, bounded by the 95% prediction intervals. (*b*) Example calibration of a hypothetical data set with fixed values of distance (±1/2 sample width) and Δ^{14}C (±1 σ error). Distances are converted to calendar years depending on a chosen growth rate. At the minimum growth rate (△), the error bar of the middle data point almost loses contact with prediction band of the reference chronology. At the maximum growth rate (▽), the oldest data point almost loses contact with the reference chronology. At the average of the minimum and maximum growth rates (■), all of the data are enclosed within the prediction bands.



^{14}C, was located <5 mm away from the internode section and was assumed to have the same age, within the dating error. From this age, growth rate and sample ages were recalculated across the radius of the node. Bomb-^{14}C chronologies from the two sites were identical, so the data were merged to produce a single reference chronology representing surface waters of the offshore branch of the Labrador Current off Newfoundland and Labrador. During the prebomb era (pre-1957), values of Δ^{14}C were stable at ~–70‰. Beginning in 1957, Δ^{14}C increased rapidly, paralleling the rise in atmospheric Δ^{14}C (Levin and Kromer 2004). Values of Δ^{14}C peaked at ~+50‰ around 1985, then decreased to ~+30‰ by the year 2000. Alternative bomb-^{14}C chronologies have been developed from otoliths of known-age fish sampled from the southwest Grand Banks (NAFO Division 3Ps) and the northern and western margins of the Labrador Sea (NAFO Divisions 0B, 1ABCDEF, 2GJ; Campana et al. 2008). In all of the chronologies, bomb ^{14}C began to increase around the year 1957; however, the signals differ in timing and magnitude of the bomb-^{14}C peak because of water mass mixing at the various sites (Sherwood et al. 2008). Therefore, our coral-based record was chosen as the reference chronology because it better reflects the slope-water region of Newfoundland and Labrador.

For each coral section to be dated, between two and four samples were isolated for Δ^{14}C, representing some combination of prebomb, bomb-increase, and postbomb data points. Calibration of Δ^{14}C data to constrain growth rates was performed using an iterative graphical procedure (Fig. 1*b*). For each coral, sample radial distances (±1/2 sample widths) were converted to calendar years (± year errors) by dividing by an estimated growth rate. Data were then plotted as Δ^{14}C vs. calendar year and compared with the reference chronology. This procedure was repeated sequentially with a range of growth rates in increments of 1 μm·year^{-1}. Minimum and maximum growth rates were constrained as the limits in which all of the data points, including error bars, remained in contact with the 95% prediction intervals enveloping the reference chronology. The average (±1/2 the range) of the minimum and maximum growth rates is reported throughout this paper. Sample age was calculated by applying the growth rate over the radius of the coral.

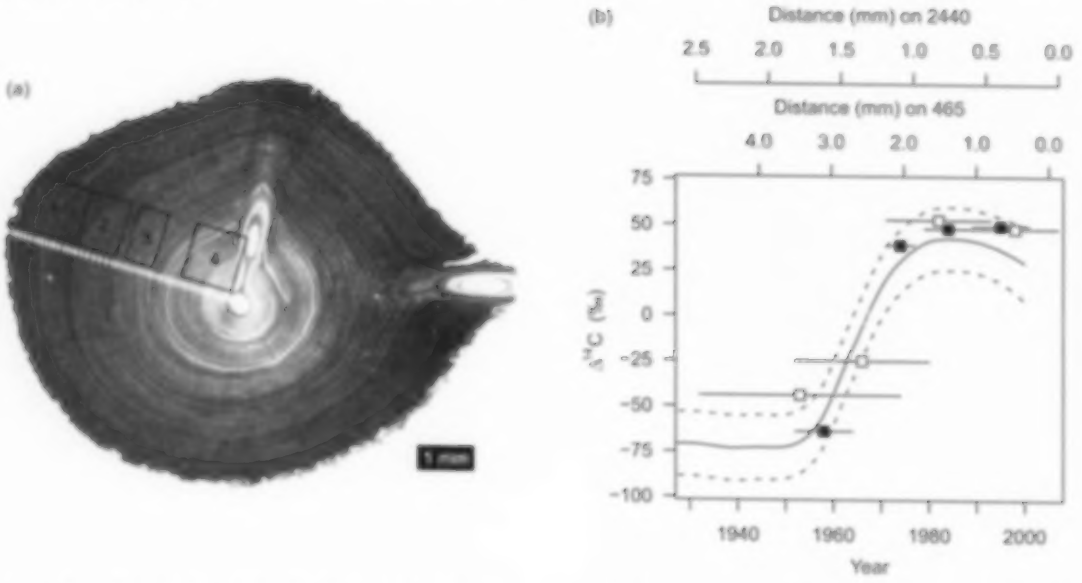
Enumeration of growth rings

With the exception of *P. arborea*, which does not exhibit growth rings, and *P. resedaeformis*, which was already known to secrete annual growth rings (Andrews et al. 2002; Sherwood et al. 2005), all of the remaining species exhibited concentric growth rings of unknown periodicity. To estimate their periodicity, ring widths were compared with radial growth rates determined using bomb-^{14}C dating. Ring widths were measured by counting the number of rings across the same radius from which samples for ^{14}C were isolated and dividing that number by the distance. In some cases, enumeration of growth rings was limited to part of the radius, because of inconsistent clarity. If the average width of growth rings matched the bomb-^{14}C-derived growth rate, within error, this was interpreted as evidence for annual periodicity of growth rings.

Results

Two colonies of *S. arctica* were examined for Δ^{14}C. This species displayed clearly visible growth rings when viewed as thick sections illuminated with ultraviolet light, or as thin sections viewed with either ultraviolet or transmitted light (Fig. 2*a*). Rings consisted of alternating light brown (luminescent) and dark brown (nonluminescent) couplets. Colony 465 was collected from the Labrador Slope at a depth of 876 m (Table 1). There were 53 growth rings, averaging 60 μm in width. Radiocarbon data constrained the radial growth rate to 66 ± 11 μm·year^{-1} and the age of the section to 55 ± 8 years (Fig. 2*b*). Another colony (2440) was collected from the southwest Grand Banks at a depth of 812 m (Table 1). In this colony, there were 58 growth

Fig. 2. (*a*) Basal thin section through the axial skeleton of *S. arctica* No. 465, illuminated with ultraviolet light. Growth rings are delineated by white hatches. Numbers 1–4 indicate the locations of samples milled for ^{14}C analysis. (*b*) The reference bomb-^{14}C chronology overlain with data for colony 465 (■) and colony 2440 (□). Sample distances are indicated along the upper horizontal axes. For colony 465, bomb-^{14}C data constrained the radial growth rate to between 55 and 76 μm·year^{-1}; data are plotted at the average of 66 μm·year^{-1}. For colony 2440, bomb-^{14}C data constrained the radial growth rate to between 22 and 44 μm·year^{-1}; data are plotted at the average of 33 μm·year^{-1}. Horizontal error bars indicate the width of individual samples converted to calendar years at indicated average growth rate. Vertical error bars are smaller than symbols.



rings, averaging 46 μm in width. Radiocarbon data constrained the radial growth rate to 33 ± 11 μm·year^{-1} and the age to 82 ± 31 years. This colony had a thinner skeletal axis, accounting for the larger errors in growth rate and age, as milled samples integrated over a wider range of growth rings. In both colonies, similarity between bomb-^{14}C-derived growth rates and rings widths provides strong evidence that the growth rings are formed annually.

A colony of *K. ornata* (No. 2452) was collected from the southern Grand Banks at a 601 m depth (Table 1). In cross section, organic nodes exhibited alternating light and dark concentric growth rings (Fig. 3*a*). Rings were easier to visualize in thick section under incident light than in thin section under transmitted light. Clarity of the rings varied from poor towards the middle of the section to good in the middle and outer regions of the section, where they averaged 80 μm in width. Four individual growth rings were isolated and analysed for $\Delta^{14}C$, which constrained the radial growth rate to 74 ± 6 μm·year^{-1} and the age to 94 ± 7 years (Fig. 3*b*). Close correspondence between the bomb-^{14}C-derived growth rate and the width of the growth rings is strong evidence that the growth rings in this species are formed annually.

A colony of *A. arbuscula* (No. 1591) was collected from the Davis Strait area at a depth of 526 m (Table 1). Skeletons of *A. arbuscula* are like those of *K. ornata*, but much smaller. Colony diameters rarely exceed 4 mm, making them difficult to sample. In cross section, the organic nodes exhibited concentric growth rings, similar to those observed in *K. ornata*, averaging 70 μm in width (Fig. 4*a*). Outer, middle, and inner regions of the node were isolated for $\Delta^{14}C$, but the inner sample was too small for analysis. Outer and middle samples produced $\Delta^{14}C$ values typical of the postbomb era. Because of this, only a minimum radial growth rate of 20 μm·year^{-1} (age < 100 years) could be determined. Based on the finding that growth rings in *K. ornata* appear to form annually, this was assumed in *A. arbuscula* as well. Using a rate of 70 μm·year^{-1} (i.e., the width of the growth rings), $\Delta^{14}C$ values plotted well within the 95% prediction intervals of the reference chronology (Fig. 4*b*). The corresponding age was 30 years. This was taken as tentative support for annual timing of growth rings in *A. arbuscula*, but more data are needed for verification.

Two colonies of *Paramuricea* spp. were examined (Table 1). Colony 1886 was collected from the central Labrador Slope at a depth of 850 m. A basal cross section exhibited seven diffuse growth rings averaging 2 mm in width (Fig. 5*a*). Radiocarbon data constrained the radial growth rate to 205 ± 20 μm·year^{-1} and the age to 71 ± 6 years. Given this age, the seven visible growth rings appear to have formed approximately once every 10 years. Another colony (No. 1348) was collected from the southwest Grand Banks at a depth of 814 m. In cross section, continuous growth rings were not observed. $\Delta^{14}C$ data constrained the radial growth rate to 92 ± 18 μm·year^{-1} and the age to 103 ± 14 years.

A large, 130 cm high colony of *P. arborea* (No. 1523) was collected from the Hudson Strait area at a depth of 414 m (Table 1). *Paragorgia arborea* has a porous texture, with the pores aligned somewhat concentrically (Fig. 6*a*);

Fig. 3. (*a*) Thick section through a basal node of *K. ornata* No. 2452. Growth rings, where visible, are delineated by white hatches. Numbered arrows point to individual rings isolated for ^{14}C analysis. (*b*) Reference bomb-^{14}C chronology overlain with data for colony 2452. Bomb-^{14}C data constrained the radial growth rate to between 68 and 79 μm·year^{-1}; data are plotted at the average of 74 μm·year^{-1}.

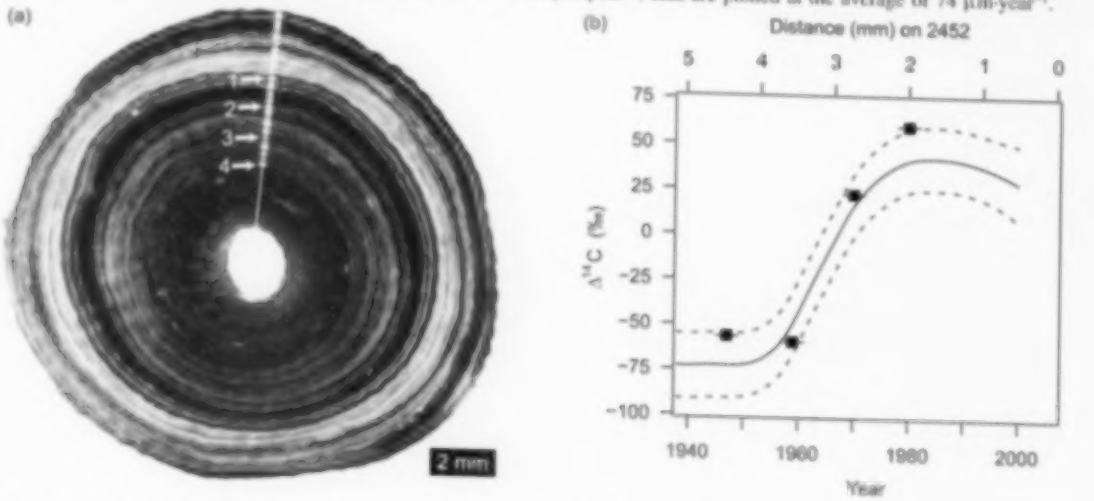
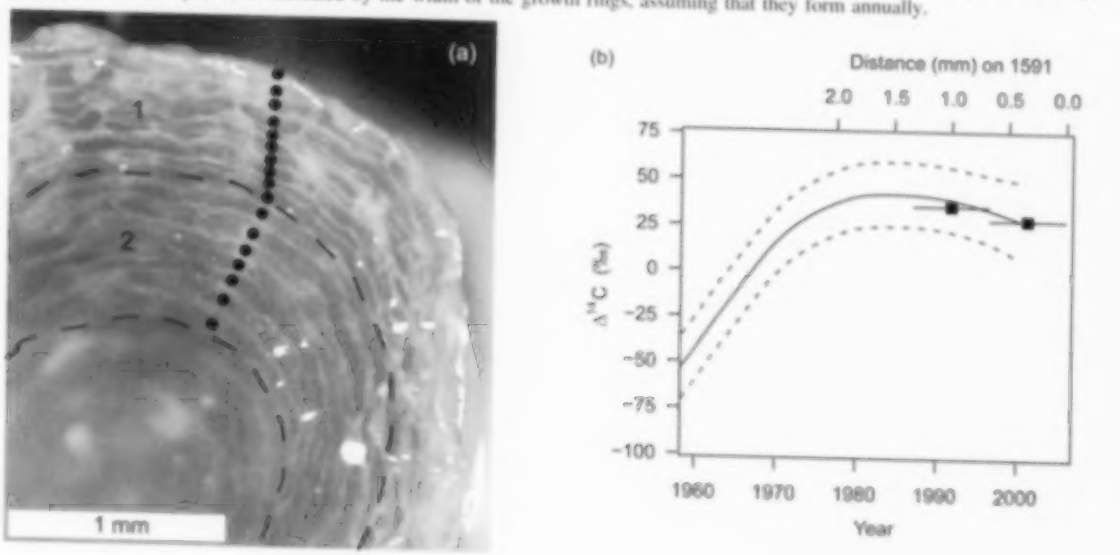


Fig. 4. (*a*) Close-up of a thick section through a basal node of *A. arbuscula* No. 2452. Numbers indicate samples for ^{14}C analysis. Growth rings are delineated by solid circles. (*b*) Reference bomb-^{14}C chronology overlain with data for colony 1591. Because only two samples were measured, $\Delta^{14}C$ did not constrain an absolute growth rate, but rather only a minimum of >20 μm·year^{-1}. Data were therefore replotted at a growth rate of 70 μm·year^{-1}, as indicated by the width of the growth rings, assuming that they form annually.



however, continuous growth rings could not be visualized, as previously reported for this species (Mortensen and Buhl-Mortensen 2005). Three samples were isolated for $\Delta^{14}C$; calibration of the middle sample to the period of bomb-^{14}C increase constrained the radial growth rate to 830 ± 120 μm·year^{-1} (Fig. 6*b*). At this growth rate, however, the oldest sample plotted slightly outside the 95% prediction intervals of the reference curve during the prebomb period. We suspect that this sample may have been contaminated with younger carbon. Alternatively, the radial growth rate may have been significantly higher during the first half of the life of the colony. Additional data points would be needed to resolve this question.

Plots of radius vs. age and axial height vs. age for all of the colonies above, with additional data for conventional ^{14}C- and ^{210}Pb-dated colonies of *K. ornata* and growth ring dated colonies of *P. resedaeformis* are shown (Fig. 7). Radial growth rates delimit the coral species into three groups (Fig. 7*a*); *S. arctica*, *K. ornata*, and *A. arbuscula* had the

Fig. 5. (*a*) Basal thick section of *Parumuricea* spp. colony 1886. Numbered segments indicate the locations of samples isolated for $\Delta^{14}C$ analysis. Solid circles indicate concentric growth rings forming with an apparent periodicity of ~10 years. (*b*) Reference bomb-^{14}C chronology overlain with data for colony 1886 (open squares) and colony 1348 (solid squares). For colony 1886, bomb-^{14}C data constrained the radial growth rate to between 185 and 224 μm·year^{-1}; data are plotted at the average of 205 μm·year^{-1}. For colony 1348, bomb-^{14}C data constrained the radial growth rate to between 74 and 110 μm·year^{-1}; data are plotted at the average of 92 μm·year^{-1}.

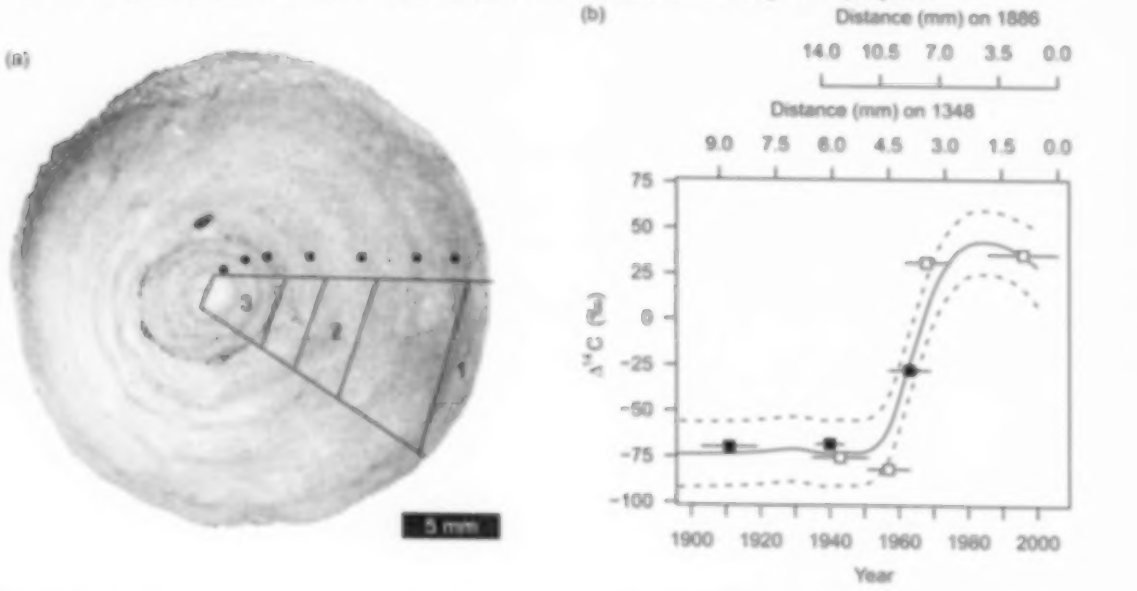
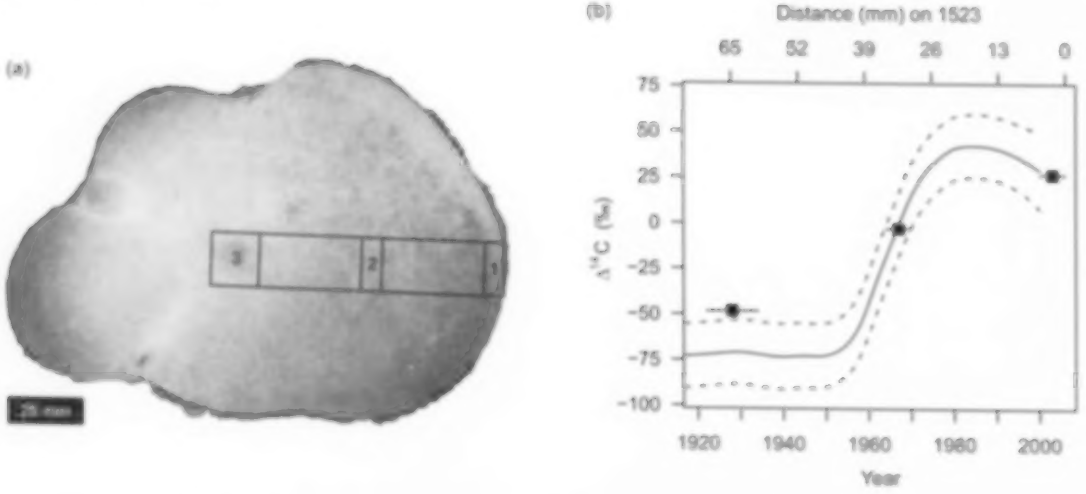


Fig. 6. (*a*) Basal thick section of *Paragorgia arborea* No. 1523. Numbered segments indicate the locations of samples isolated for $\Delta^{14}C$ analysis. Note skeletal porosity and lack of visible growth rings. (*b*) Reference bomb-^{14}C chronology overlain with data for colony 1523. Bomb-^{14}C data constrained the radial growth rate to between 710 and 950 μm·year^{-1}; data are plotted at the average of 830 μm·year^{-1}. The oldest data point plots slightly outside the 95% prediction intervals of the reference chronology, perhaps indicating reworking of the skeletal matrix and (or) nonlinear radial growth.



lowest growth rates, ranging from 33 ± 11 to 75 ± 11 μm·year^{-1}. *Parumuricea* spp. and *P. resedaeformis* had faster radial growth rates, ranging from 83 ± 6 to 215 ± 37 μm·year^{-1}. Finally, *P. arborea* thickens much faster than any of the other species, at 830 ± 120 μm·year^{-1}. Axial growth rates ranged from 0.56 ± 0.05 cm·year^{-1} for *Parumuricea* spp. to 2.61 ± 0.45 cm·year^{-1} for *P. resedaeformis* (Fig. 7*b*).

Plots of radius vs. age and axial height vs. age for two different populations of *P. resedaeformis* collected from the Hudson Strait (Table 1) and the Northeast Channel, southwest of Nova Scotia (Mortensen and Buhl-Mortensen 2005)

Fig. 7. (*a*) Skeletal radius vs. age for deep-sea antipatharians and gorgonians off Newfoundland and Labrador: *Stauropathes arctica*, ■; *Keratoisis ornata*, ▲; *Acanella arbuscula*, ▼; *Paramuricea* spp., □; *Paragorgia arborea*, △; *Primnoa resedaeformis*, ▽. Reference lines indicate growth rates of 50 and 150 μm·year^{-1}. (*b*) Axial height vs. age for the same colonies (where available). Reference lines indicate axial growth rates of 0.5 and 2 mm·year^{-1}.

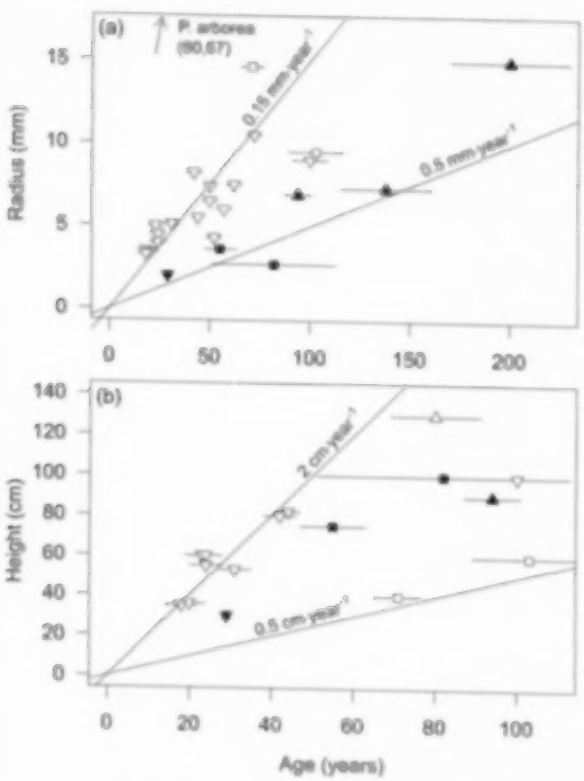
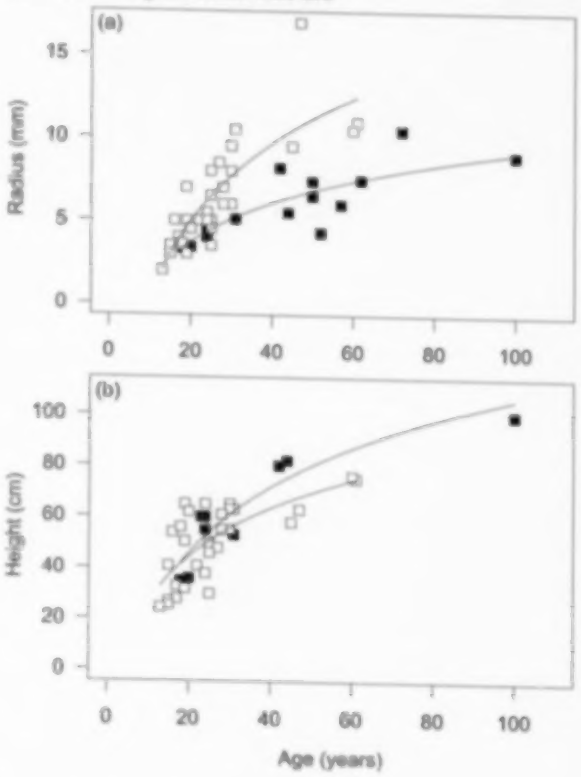


Fig. 8. Comparison of (*a*) radial and (*b*) axial growth rates in colonies of *Primnoa resedaeformis* collected from the Hudson Strait (this study; ■) and the Northeast Channel (Mortensen and Buhl-Mortensen 2005; □). In both cases, logarithmic fits were superior to linear fits, as indicated by r^2 values. Radial growth rates were significantly higher at the Northeast Channel than at the Hudson Strait, but axial growth rates were not.



are shown (Fig. 8). The Northeast Channel population had a significantly higher radial growth rate (230 ± 60 μm·year^{-1}) than the Hudson Strait population (151 ± 39 μm·year^{-1}; *t* test, $p < 0.001$; Fig. 8*a*), but there was no significant difference in axial growth rates between the two populations (Fig. 8*b*).

Discussion

The presence of bomb radiocarbon in all of the species examined here adds further support to the theory that recently fixed and exported POM is the primary source of carbon to the organic endoskeletons of deep-sea gorgonians and antipatharians. Bomb-^{14}C dating relies on the assumption that there is no phase-lag or signal attenuation with depth. Relative to surface water bomb-^{14}C chronologies, deep-water chronologies derived from the skeletal carbonate of deep-sea corals (Sherwood et al. 2008) and from otoliths of deep-dwelling fish (Campana et al. 2008) are both lagged and attenuated. Maximum levels of bomb ^{14}C in our unknown-age corals did not appear attenuated with respect to the reference chronology, perhaps with the exception of *A. arbuscula* and *P. arborea*, for which more data were needed to resolve the bomb peak. Therefore, we conclude that it is unlikely that bomb-^{14}C data from unknown-age colonies were phase-lagged. Consequently, the bomb-^{14}C dating method provided robust estimates of growth rates and ages. Errors averaged 20%. It is doubtful that this error could be much improved, even with tighter sampling across the skeletons, because of the variability in radial growth rates evident in the asymmetric shape of skeletal cross sections. For example, the difference between maximum and minimum radial growth rates ranged from 14% for *K. ornata* (No. 1343) to 52% for *P. arborea* (No. 1523).

Bomb-^{14}C dating may be compromised where younger carbon is exchanged with older parts of the skeleton, for example, by the actions of boring organisms (Boerboom et al. 1998) or by secondary ingrowth around the central core of the skeletal axis (Lewis et al. 1992; Tracey et al. 2007). This may have been the case with the innermost sample of *P. arborea* (No. 1523). High skeletal porosity in this species may facilitate the reworking of the skeletal matrix (Andrews

et al. 2005). *Paramuricea* spp. also forms a spongy axis without clearly delineated growth rings, but there was no indication of reworking. The remaining species have consolidated axes that are much more resistant to reworking.

Concentric growth rings in gorgonian and antipatharian corals are often assumed to form annually, but this is rarely validated for particular species and locations using independent dating methods. In addition to *P. resedaeformis* growing off eastern Canada (Sherwood et al. 2005), bomb radiocarbon data show that three additional species (*A. arbuscula*, *K. ornata*, and *S. arctica*) also secrete annual growth rings in their axial skeletons. *Acanella arbuscula* and *K. ornata* are in the same family (Isididae), so it is not surprising that they exhibit similar growth ring morphology and periodicity. In both species, growth rings were visualized in the organic nodes of the skeletal axes and consisted of alternating light brown and dark brown couplets. Color variation may have to do with differences in protein tanning, as suggested for shallow-water gorgonians (Szmant-Froelich 1974), but this remains untested. Unfortunately, inconsistent clarity of growth rings may limit their use in dating colonies of *A. arbuscula* and *K. ornata* without independent validation. Growth rings in the calcite internodes, as opposed to the organic nodes studied here, of Isidid corals have also been described, but poor clarity and unclear periodicity make them unreliable as an indicator of colony age (Thresher et al. 2004; Andrews et al. 2005; Tracey et al. 2007). Roark et al. (2005), however, were successful in counting growth rings in the calcitic internodes of Alaskan Isidids. Growth rings in *S. arctica* were of more consistent clarity. Use of ultraviolet light improved ring discrimination tremendously because the growth rings consist of alternating layers of chitin (fluorescent) and nonfibrillar protein (nonfluorescent; Kim et al. 1992). Clarity of growth rings in this species suggests that this could be used to date large numbers of colonies relatively quickly and inexpensively. In contrast, growth rings in *Paramuricea* spp. did not form annually and should not be relied upon to date colonies (cf. Mistri and Ceccherelli 1994), whereas growth rings in *P. arborea* could not be visualized at all.

Growth rates of Newfoundland and Labrador corals match very closely with growth rates in related taxa from different regions of the world. The antipatharian *S. arctica* had the lowest radial growth rate of 33 ± 11 to 66 ± 11 μm·year^{-1}. Antipatharians, in general, are some of the slowest-growing deep-sea corals, with radial growth rates of ~15 μm·year^{-1} for *Leiopathes glaberrima* collected off Florida, USA (Williams et al. 2006) and ≤10 μm·year^{-1} (age > 2000 years) for *L. glaberrima* collected off Hawaii (Roark et al. 2006). Isidid corals (*A. arbuscula*, *K. ornata*) had similarly low radial growth rates of 53 ± 9 to 75 ± 11 μm·year^{-1}. Similar values were reported for bamboo corals collected off California (50–110 μm·year^{-1}; Andrews et al. 2005), the Gulf of Alaska (50–60 μm·year^{-1}; Roark et al. 2005), Tasmania (~100 μm·year^{-1}; Thresher et al. 2007), and New Zealand (130–290 μm·year^{-1}; Tracey et al. 2007). Radial growth rates for *P. arborea* have not been reported previously using radiometric techniques; however, axial growth rates have been estimated at 0.8–4 cm·year^{-1} for *P. arborea* specimens from New Zealand (Tracey et al. 2003) and Norway (Mortensen and Buhl-Mortensen 2005), similar to the 1.62 ± 0.22 cm·year^{-1} in our Hudson Strait specimen. To our knowledge, these are the first estimates of radial (92 ± 18 μm·year^{-1} to 205 ± 20 μm·year^{-1}) and axial (0.56 ± 0.05 μm·year^{-1} to 0.58 ± 0.08 cm·year^{-1}) growth rates for deep-sea *Paramuricea* spp.; shallow-water congeners grow at slightly faster axial growth rates (1.8 cm·year^{-1}; Coma et al. 1998).

Similarities and differences in growth rates between species may reflect, in part, mechanical requirements of skeletal axes. For example, *P. resedaeformis* and *P. arborea* inhabit relatively shallow, faster-current environments. *Primnoa resedaeformis* forms stiff, consolidated axes. The weaker, unconsolidated axes of *P. arborea* require a greater degree of axial thickening to withstand the same current velocities. By contrast, *S. arctica*, Isidids, and *Paramuricea* spp. all mostly inhabit relatively deeper, slower-current environments. *Stauropathes arctia* and Isidids form stiff axes requiring less axial thickening than the weaker, spongy axes of *Paramuricea* spp. in a similar a current regime.

Differences in radial growth rates between populations of the same species may reflect adaptations to local current regimes. For example, there was a significant difference in radial growth rates among Northeast Channel and Hudson Strait populations of *P. resedaeformis*. Faster radial growth rates in the Northeast Channel population may be a response to the strong tidal currents, which average 100 cm·s^{-1} in this submarine canyon (current meter data retrieved from area SS29 in the Ocean Data Inventory, available online at http://www.mar.dfo-mpo.gc.ca/science/ocean/database/data_query.html). The Hudson Strait population was sampled near the Hatton Basin, a large, bowl-shaped bathymetric depression to the east of Hudson Strait, where current velocities average only 40 cm·s^{-1}. Lack of difference in axial growth rates between these two populations suggests that axial growth rates may be less sensitive to the influence of current velocities than radial growth rates.

Overall, our results show that deep-sea corals off Newfoundland and Labrador are slow-growing and long-lived. All of the specimens reported on here were collected from heavily fished areas, with the exception of the Hudson Strait (Edinger et al. 2007*b*). The largest and oldest colonies are likely to have already been removed from the population, thereby biasing age distributions toward smaller and younger colonies (cf. Anderson and Clarke 2003). This may explain why exceptionally large and old colonies (>100 years) collected off eastern Canada are usually subfossil in age. Little is known about the frequency of recruitment events in deep-sea gorgonians and antipatharians; however, knowledge of shallow-water taxa (Grigg 1977; Farrant 1987; Lasker et al. 1998) and preliminary evidence from deep-sea species (Mills and Mullineaux 2005) suggest that it may be infrequent. The combination of great longevity, slow growth rates, and infrequent recruitment implies that populations of deep-sea corals damaged by fishing gear will take centuries to recover. Conservation measures to protect deep-sea corals off Newfoundland and Labrador should be designed and implemented immediately.

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Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited effective dispersal in the northeastern Pacific Ocean

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Abstract: Genetic population structure of Pacific cod, *Gadus macrocephalus*, was examined across much of its northeastern Pacific range by screening variation at 11 microsatellite DNA loci. Estimates of F_{ST} (0.005 ± 0.002) and R_{ST} (0.010 ± 0.003) over all samples suggested that effective dispersal is limited among populations. Genetic divergence was highly correlated with geographic distance in an isolation-by-distance (IBD) pattern along the entire coastal continuum in the northeastern Pacific Ocean (~4000 km; $r^2 = 0.83$), extending from Washington State to the Aleutian Islands, and over smaller geographic distances for three locations in Alaska (~1700 km; $r^2 = 0.56$). Slopes of IBD regressions suggested average dispersal distance between birth and reproduction of less than 30 km. Exceptions to this pattern were found in samples taken from fjord environments in the Georgia Basin (the Strait of Georgia (Canada) and Puget Sound (USA)), where populations were differentiated from coastal cod. Our results showed population structure at spatial scales relevant to fisheries management, both caused by limited dispersal along the coast and by sharp barriers to migration isolating smaller stocks in coastal fjord environments.

Résumé : Nous avons examiné la structure génétique de la population des morues du Pacifique, *Gadus macrocephalus*, dans presque toute son aire de répartition dans le nord-est du Pacifique, par la détermination de la variation à 11 locus microsatellites d'ADN. Les valeurs estimées de F_{ST} (0,005 ± 0,002) et R_{ST} (0,010 ± 0,003) pour l'ensemble des échantillons laissent penser que la dispersion entre les populations est limitée. Il y a une forte corrélation entre la divergence génétique et la distance géographique selon un patron d'isolement par la distance (IBD) sur l'ensemble du continuum côtier dans le nord-est du Pacifique (~4000 km; $r^2 = 0,83$), depuis l'état de Washington aux îles Aléoutiennes, ainsi que sur des distances plus courtes dans trois sites d'Alaska (~ 700 km; $r^2 = 0,56$). Les pentes des régressions d'IBD indiquent que la distance moyenne de dispersion de la naissance à la reproduction est inférieure à 30 km. Des exceptions à ces patrons se retrouvent dans les échantillons provenant des environnements de fjords dans le bassin de Géorgie, soit le détroit de Géorgie (Canada) et Puget Sound (É.-U.), où les populations se différencient des morues de la côte. Nos résultats présentent une structure de population à des échelles qui sont d'intérêt pour la gestion des pêches, causée à la fois par une dispersion restreinte le long de la côte et par d'importantes barrières à la migration qui isolent les stocks plus petits dans les environnements des fjords côtiers.

[Traduit par la Rédaction]

Introduction

Although the notion that exploited species should be managed at the population level can be traced back to seminal work by Heincke (1889) and Hjort (1914), the practical implementation of such insight is still fraught with difficulties. Population boundaries are often ill defined by environmental, biological, or genetic discontinuities, especially in marine fishes (Hauser and Ward 1998). The result is that demographically independent populations may appear genetically homogenous and thus may be managed as a single stock. Most genetic approaches to population identification are further limited by their lack of sensitivity to high levels of connectivity and their dependence on the number of migrants rather than migration rates, complicating their application for the identification of independent management units (Waples and Gaggiotti 2006). This discrepancy between genetic and demographic definitions of populations

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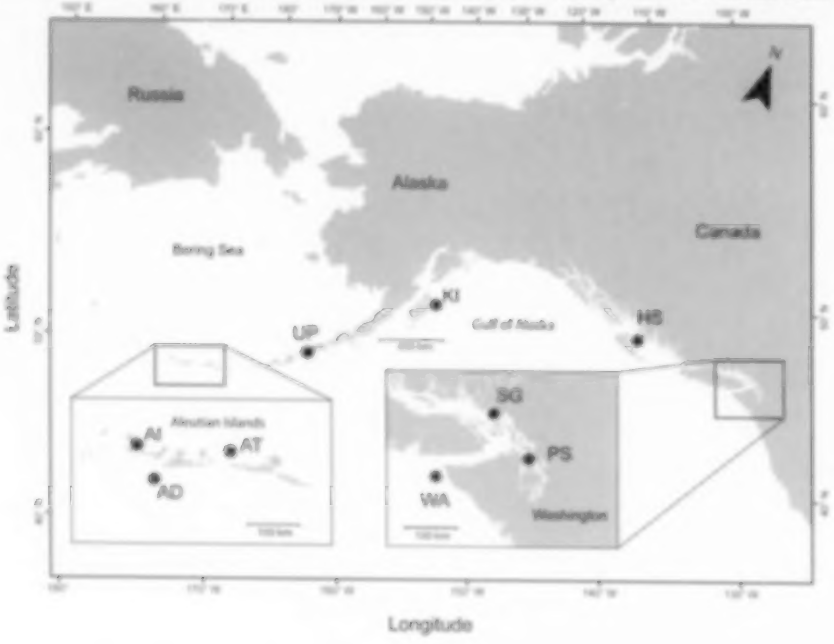
Fig. 1. Sample locations for Pacific cod (*Gadus macrocephalus*). Sample abbreviations are central Aleutian Islands (AI, AD, and AT), Unimak Pass (UP), Kodiak Island (KI), Hecate Strait (HS), coastal Washington State (WA), Puget Sound (PS), and Strait of Georgia (SG).



means that genetic estimates of population structure tend to be very conservative in marine species; genetic differentiation demonstrates population structure, but genetic homogeneity does not preclude the existence of demographically independent populations (Shaklee and Bentzen 1998).

Recently, the explicit inclusion of geographic information in genetic analyses has improved our ability to identify populations as management units. The search for sharp population boundaries may be futile in marine species, which often are distributed continuously and may disperse during all life history stages. Instead, the dispersal of individuals within that continuous distribution may be limited, resulting in a continuous increase of genetic differentiation with geographic distance (isolation by distance, IBD). Under certain assumptions (*e.g.*, migration-drift equilibrium, exponential dispersal curve), the slope of the relationship can be used to estimate average dispersal distance of individuals (Rousset 1997). Although not providing stock boundaries, such estimates can be used in models to define geographic sectors of the fishery that may respond independently to exploitation.

IBD has been primarily described in demersal species of fish with pelagic larvae, e.g., species of the family Gadidae. In particular, several studies on Atlantic cod (*Gadus morhua*) showed IBD (Pogson et al. 2001; Beacham et al. 2002; O'Leary et al. 2007), whereas other studies identified patchy population structure that was not as clearly correlated with geographic distribution (e.g., Hutchinson et al. 2001; Knutsen et al. 2003). Furthermore, IBD was primarily identified when high variability markers, such as mitochondrial DNA and especially microsatellites, were applied. Pacific gadid species such as walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) are relatively understudied in this respect, despite their high economic importance (Hiatt et al. 2007), and knowledge on their stock structure lags behind that for Atlantic cod by perhaps a decade or more (Cunningham 2007).

Pacific cod has historically supported large- and small-scale commercial fisheries throughout its range. Global catch landings in 2006 were 235 296 tonnes (t), and in the United States, Pacific cod catches exceeded those of declining Atlantic cod stocks by the mid-1980s (http://www.st.nmfs.noaa.gov/st1/commercial/index.html). Although numerous putative stocks of Pacific cod have been identified using a variety of methods (see review in Gustafson et al. 2000), no general consensus has been reached on stock structure and Alaskan fisheries are managed as two separate stocks: the Gulf of Alaska (GOA) stock and the much larger Bering Sea – Aleutian Islands (BSAI) stock (Thompson et al. 2007*a*, 2007*b*). The largest Pacific cod fishery in Alaska (average catch 1990–2007 = 156 482 t) occurs in the eastern Bering Sea (Thompson et al. 2007*a*). Smaller fisheries, representing approximately 16% of the total harvest in the BSAI management area, occur in the Aleutian Islands region. Near the southern end of the species' distribution, populations have declined rapidly in the Georgia Basin, which consists of the Strait of Georgia, British Columbia, Canada, and fjord-like estuary of Puget Sound, Washington State, USA (Fig. 1). These two regions are directly connected to the North Pacific Ocean via the Johnstone Strait and the Strait of Juan de Fuca, respectively.

Here we present an assessment of population structure in Pacific cod inferred from microsatellite DNA variation across much of its North American range. The results demonstrated a clear IBD pattern, suggesting restricted gene

Table 1. Sampling locations, abbreviations, dates, and numbers (n) for adult Pacific cod (*Gadus macrocephalus*).

Geographic location, state or province	Sample abbreviation	Collection agency[a]	Date (month/year)	Latitude	Longitude	n
Adak Island, Alaska	AD	NMFS	3/2006	51°40′N	176°30′W	45
Central Aleutian Islands, Alaska	AI	NMFS	2/2005	51°50′N	177°36′W	92
Atka Island, Alaska	AT	NMFS	4/2006	52°18′N	173°38′W	45
Unimak Pass, Alaska	UP05	NMFS	1/2005	54°38′N	168°10′W	87
Unimak Pass, Alaska	UP03	NMFS	1/2003	54°38′N	168°10′W	95
Kodiak Island, Alaska	KI05	UW	3/2005	57°55′N	152°18′W	106
Kodiak Island, Alaska	KI03	NMFS	3/2003	57°48′N	152°31′W	94
Hecate Strait, British Columbia, Canada	HS	DFO	3/2004	53°13′N	130°57′W	89
Coastal Washington, Washington	WA	UW	2/2005	47°55′N	125°33′W	69
Strait of Georgia, Washington	SG	WDF&W	4/2003	48°54′N	123°06′W	94
Puget Sound, Washington	PS	WDF&W	2/2004, 3/2006	47°32′N	122°30′W	18

[a]NMFS, National Marine Fisheries Service; UW, University of Washington, School of Aquatic and Fisheries Science; DFO, Fisheries and Oceans Canada; WDF&W, Washington Department of Fish and Wildlife.

flow, and thus a substantial amount of self-recruitment, among putative stock components at spatial scales relevant to current fisheries management and conservation practices. In particular, Pacific cod (like Atlantic cod) appear to form localized populations in fjord environments or where deep-water barriers, such as submarine canyons, may limit adult dispersal.

Materials and methods

Sample collection

Samples were collected from large spawning and pre-spawning aggregates of Pacific cod in eight locations across the northeastern Pacific Ocean from January–March (Table 1; Fig. 1). Samples were primarily taken from trawls, although those from Kodiak Island in 2005 were caught with jigging gear. Replicate samples were taken at two-year intervals at two locations (Unimak Pass (UP03 and UP05) and Kodiak Island (KI03 and KI05), Alaska) to investigate temporal stability of population structure. Two samples from the central Aleutian Islands region (AD and AT), taken during commercial fishing operations in 2006, were in relatively close proximity (180 and 275 km, respectively) to one sample collected during a trawl survey in 2005 (AI). Samples obtained from Puget Sound in 2004 and 2006 were pooled because of small sample sizes. Pectoral fin clips were preserved in 95% ethanol and stored at room temperature prior to DNA extraction.

DNA extraction and microsatellite amplification

DNA was extracted from pectoral fin tissue using Qiagen DNeasy kits (QIAGEN, Valencia, California) following the manufacturer's protocols. Twelve microsatellite markers were initially screened for variation: 10 novel loci isolated from Pacific cod (Canino et al. 2005), one (*GMO37*) isolated from Atlantic cod (Miller et al. 2000), and one (*TCH20*) from walleye pollock (O'Reilly et al. 2000). Polymerase chain reaction (PCR) amplification of loci was conducted in 10 μL reaction volumes containing 4 μL template DNA, 1 μL 10× buffer (Genechoice Inc., Fredrick, Maryland), $MgCl_2$ concentration of 1.5 mmol·L^{-1}, 0.4 mmol·L^{-1} of each dNTP, 0.5 mmol·L^{-1} labeled forward and reverse primers, and 0.5 units of *Taq* Polymerase (Genechoice Inc., Fredrick, Maryland). A single "touchdown" thermal cycle for amplifying all loci was performed in a PTC-100 thermal cycler (MJ Research Inc., Waltham, Massachusetts). This consisted of an initial denaturation step at 95 °C (2 min) followed by five cycles of 95 °C (1 min), 60 °C (1 min (–1 °C per cycle)), and 72 °C (1 min) and then by 20 cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (30 s). Microsatellite PCR amplicons were size-separated in four panels of three loci each on a MegaBACE 1000 DNA sequencer (Amersham Pharmacia Biotech, Inc., Piscataway, New Jersey) and analyzed with Genetic Profiler software (version 2.2, Amersham Pharmacia Biotech).

Data analysis

Single locus statistics for all samples (Appendix A, Table A1) were calculated using FSTAT 2.9.3.2 (Goudet 2002) and Genetix 4.05 (Belkhir 2000). Genotype frequency conformance to Hardy–Weinberg equilibrium (HWE) and genotypic linkage equilibrium were determined using exact tests implemented in GENEPOP 3.3 (Raymond and Rousset 1995) with Markov chain parameters of 5000 dememorization steps, 500 batches, and 5000 iterations per batch. Significance levels for comparisons of loci across samples were adjusted using a sequential Bonferroni correction (Rice 1989) to maintain a type I error rate ($\alpha = 0.05$) over multiple tests. The program MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004) was used to check for the presence of null alleles, scoring errors, stuttering, and large allele dropout.

Exact tests for genic and genotypic differentiation for each locus–sample combination were conducted in GENEPOP using the same Markov chain Monte Carlo (MCMC) parameters used to test for HWE. In addition, we tested the joint null hypothesis of no frequency differences at any locus for each sample using Fisher's combined test implemented in GENEPOP. Randomization tests permuting either alleles or genotypes among samples were conducted in FSTAT to examine differentiation between sample pairs.

Genetic differentiation (F_{ST}) over all samples and between sample pairs was determined in GENEPOP from variances in allele frequencies using the unbiased estimator θ (Weir and Cockerham 1984). The analog statistic based on allele size (R_{ST}; Slatkin 1995) was calculated over all samples and between sample pairs using SPAGeDi 2.1 (Hardy

Table 2. Results from analysis of molecular variance (AMOVA) for sample groupings containing (n) samples.

Grouping scheme	Groups	n	F_{ST}	F_{SC}	F_{CT}
All samples					
(Coastal samples) (SG,PS)	2	11	0.0116***	0.0010***	0.0094**
All samples excluding SG and PS					
(AD, AT, AI, UP03, UP05, KI03, KI05) (HS, WA)	2	9	0.0038***	0.0012**	0.0026*
(AD, AT, AI) (UP03, UP05, KI03, KI05) (HS, WA)	3	9	0.0029***	0.0005ns	0.0025***
(AD, AT, AI) (UP03, UP05) (KI03, KI05) (HS, WA)	4	9	0.0025***	0.0003ns	0.0021**
Alaska only					
(AD, AT, AI) (UP03, UP05) (KI03, KI05)	3	7	0.0010*	–0.0004ns	0.0014*
(AD, AT, AI, UP03, UP05) (KI03, KI05)	2	7	0.0012*	0.0002ns	0.0009ns
(AD, AT, AI) (UP03, UP05, KI03, KI05)	2	7	0.0015*	–0.0001ns	0.0017*
(AD, AT, AI, KI03, KI05) (UP03, UP05)	2	7	0.0008*	0.0009*	–0.0001ns

Note: Coastal samples: AD, AT, AI, UP03, UP05, KI03, KI05, HS, WA; F_{ST}, variance of groups relative to total; F_{SC}, variance among samples within groups; F_{CT}, variance of samples among groups. P values for variance components: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant. Sample abbreviations are as in Table 1.

and Vekemans 2002). We then tested for significant differences between the two estimators to examine the relative effects of genetic drift (F_{ST}) and mutation (R_{ST}) on genetic differentiation using 5000 permutations of allelic sizes among allelic states (Hardy et al. 2003).

Analysis of molecular variation (AMOVA) was conducted in Arlequin 3.11 (Excoffier et al. 2005) to test population structure across the entire geographic sample range and among putative regional groupings of samples. Correlations between pairwise estimates of F_{ST} and R_{ST} with the shortest geographic distance between samples along continental margins (<200 m) were assessed using Mantel's test with 1000 permutations of linearized F_{ST} and R_{ST} values and distance matrices with the Isolde subroutine implemented in GENEPOP. A decomposed regression analysis (Koizumi et al. 2006) was performed to detect potential outliers in the pattern of genetic and geographic distances. Means and 95% confidence intervals of residuals from the linear regression of F_{ST} on geographic distance were calculated for each sample, and putative outliers were identified if the confidence interval excluded zero. These were sequentially removed and the subsequent fit of the data to the regression model was evaluated using Akaike's information criterion following Koizumi et al. (2006).

Mean dispersal distance per generation, σ, was calculated from the slope of the IBD regression relationship following Buonaccorsi et al. (2005):

$$\sigma = \sqrt{(8Dm)^{-1}}$$

where D is density of fish per linear kilometre (km), and m is the slope of the least-squares regression of $F_{ST}/(1 - F_{ST})$ on geographic distance. This one-dimensional model is a reasonable choice for estimating dispersal when differentiation occurs over spatial scales greater than the habitat width (Rousset 1997) and seems generally appropriate for broadly distributed species confined to continental margins. We assumed that the dispersal pattern followed a symmetrical, exponential distribution and calculated mean dispersal distances for effective population densities ranging from 1 to 100 000 fish·linear km^{-1} of coastline.

The program Barrier 2.2 (Manni et al. 2004) was used to identify genetic discontinuities within the general IBD pattern. The program uses Delaunay triangulation to calculate a geometric network of samples connected by F_{ST} values. Using a Monmonier algorithm, the program then identifies the edge with the largest F_{ST} given the geographic distance and extends that barrier to the next connection with the largest genetic distance until it hits another edge or another barrier. One such barrier was calculated for each locus. In addition, a spatial analysis of molecular variance (SAMOVA) developed by Dupanloup et al. (2002) was conducted to define groupings of samples that were maximally differentiated from each other. Samples were randomly partitioned into two to five groups for 100 simulated annealing processes. The configuration producing the largest among-group variance component (F_{CT}) was chosen as the best spatial pattern defining the population structure.

Results

Sample sizes for single locus statistics ranged from 11 to 106 individuals per locus (Appendix A, Table A1). Observed heterozygosities ranged from 0.235 (*GMA108*) to 1.000 (*TCH20*). Locus *GMA107* did not meet Hardy–Weinberg equilibrium (HWE) expectations because of significant homozygote excesses in most samples. Results from MICRO-CHECKER inferred evidence for null alleles and this locus was excluded in subsequent analyses.

Twelve of 121 sample–locus combinations showed significant deviations from HWE expectations, with no apparent trends within loci or samples, and only one locus (*GMA106*) in the Aleutian Islands (AI) sample remained significant after Bonferroni correction (Appendix A, Table A1). Linkage disequilibrium was detected between two loci (*GMA101* and *GMO37*) in one sample from the central Aleutian Islands (AD) but was not significant in pairwise tests in any other sample or over all samples.

Hierarchical AMOVA was used to explore several potential geographic subdivisions by partitioning samples into two to four groups (Table 2). The highest F_{ST} values were found when the samples were partitioned into coastal North Pacific and Georgia Basin groups, but this also resulted in a highly significant within-group variance component (F_{SC}). Partitioning the coastal samples into three or four groups eliminated significant F_{SC} results and produced approximately

Table 3. Probability (*P*) values from exact tests of genic (above diagonal) and genotypic (below diagonal) differentiation between sample pairs.

	AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS
AD	—	0.7128	0.6908	0.0976	0.6367	0.0150*	0.0186*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
AI	0.6186	—	0.7261	0.0120*	0.1470	0.0455*	0.0603	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
AT	0.6367	0.7442	—	0.5001	0.5291	0.0640	0.1453	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
UP05	0.3139	0.0555	0.8279	—	0.5889	0.3385	0.4042	**<0.0001**	**0.0014**	**<0.0001**	**<0.0001**
UP03	0.4532	0.1742	0.4378	0.4470	—	0.6444	0.1207	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**
KI05	0.0288*	0.1514	0.0958	0.3460	0.5492	—	0.7159	**<0.0001**	**0.0009**	**<0.0001**	**<0.0001**
KI03	0.0137*	0.1681	0.2375	0.4605	0.1746	0.7669	—	**0.0011**	0.0101*	**<0.0001**	0.0060*
HS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0042*	—	0.0104*	**<0.0001**	0.0121*
WA	**<0.0001**	**<0.0001**	**<0.0001**	0.0041*	**<0.0001**		0.0259*	0.0215*	—	**<0.0001**	0.0180*
SG	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	—	0.3778
PS	0.0002*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0039*	0.0080*	0.0083*	0.2758	—

Note: Sample abbreviations are as in Table 1. Values in bold indicate sample pairs significantly differentiated following sequential Bonferroni correction for 55 multiple tests (initial $\alpha = 0.00091$). An asterisk (*) denotes significant prior to sequential correction.

Table 4. Estimates of F_{ST} (above diagonal) and R_{ST} (below diagonal) between sample pairs.

	AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS
AD	—	–0.0012	0.0000	0.0017	–0.0004	0.0026	0.0018	**0.0060**	**0.0061**	**0.0142**	0.0215
AI	–0.0040	—	0.0002	0.0024	0.0016	0.0022	0.0018	**0.0065**	**0.0062**	**0.0163**	0.0247*
AT	–0.0067	–0.0058	—	–0.0004	0.0001	0.0018*	0.0019	**0.0037**	**0.0063**	**0.0105**	0.0183*
UP05	0.0011	–0.0012	–0.0031	—	0.0001	0.0004	0.0004	**0.0036**	0.0031*	**0.0109**	0.0152*
UP03	–0.0003	–0.0017	–0.0028	–0.0017	—	0.0000	0.0001	0.0036*	**0.0045**	**0.0120**	0.0179*
KI05	–0.0036	–0.0008	–0.0056	–0.0007	0.0014	—	–0.0006	**0.0026**	**0.0029**	**0.0101**	0.0157*
KI03	–0.0057	–0.0012	–0.0062	0.0030	0.0020	–0.0025	—	0.0027*	0.0028*	**0.0114**	0.0142*
HS	0.0093	0.0024	0.0033	0.0037	0.0083	0.0071	0.0070	—	0.0030*	**0.0058**	**0.0112**
WA	–0.0028	–0.0016	–0.0036	0.0058	0.0029	0.0031	–0.0018	0.0008	—	**0.0072**	0.0097
SG	0.0195	0.0232	0.0179	0.0271	0.0331	0.0217	0.0190	0.0105	0.0103	—	0.0024
PS	0.0370	0.0251	0.0299	0.0342	0.0383	0.0364	0.0307	0.0031	0.0049	–0.0071	—

Note: Sample abbreviations are as in Table 1. F_{ST} values in bold are significant following sequential Bonferroni correction for 55 multiple tests (initial $\alpha = 0.00091$); asterisk (*) denotes significant prior to sequential correction.

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Table 5. Isolation-by-distance (IBD) regression statistics for estimates of F_{ST} with geographic distance among groupings of North American samples of Pacific cod (*Gadus macrocephalus*).

Samples excluded	n	Slope	r^2	P	AIC_C	ΔAIC_C
None	11	3.10×10^{-6}	0.47	9.4×10^{-9}	–90.83	15.86
PS	10	2.31×10^{-6}	0.53	1.6×10^{-8}	–92.87	13.82
PS, SG	9	1.57×10^{-6}	0.83	1.2×10^{-14}	–106.69	0.00
PG, SG, WA, HS (Alaska only)	7	1.38×10^{-6}	0.56	9.6×10^{-5}	–87.14	19.55

Note: Sample abbreviations as in Table 2. Akaike information criterion values corrected for small sample sizes (AIC_C) and difference from the best model (ΔAIC_C) were calculated from decomposed pairwise regression analysis.

Fig. 2. Linearized F_{ST} versus geographic distance for Pacific cod (*Gadus macrocephalus*). Regression line is fitted to data from coastal samples in North America (solid circles). Open diamonds and solid squares represent data from the Strait of Georgia and Puget Sound, respectively.

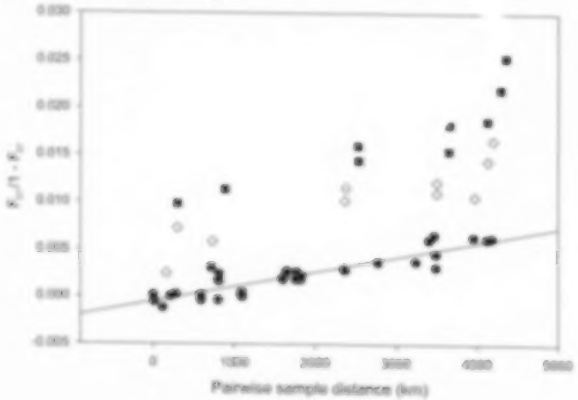


Fig. 3. Means and 95% confidence intervals of individual sample residuals from the regression of linearized F_{ST} on geographic distance over all other samples. Sample abbreviations as in Table 1.

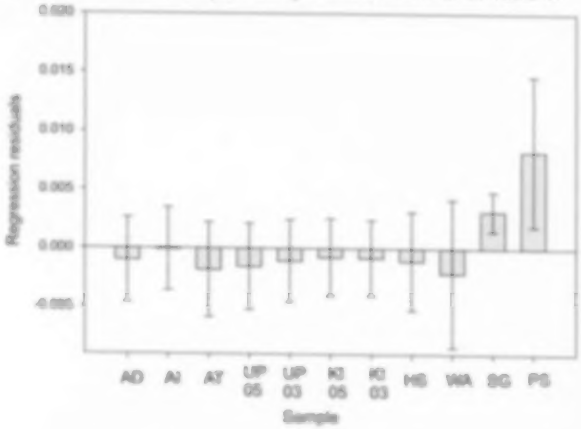


Table 6. Estimated mean dispersal distances for Pacific cod (*Gadus macrocephalus*) based on isolation-by-distance regression over a range of effective adult densities.

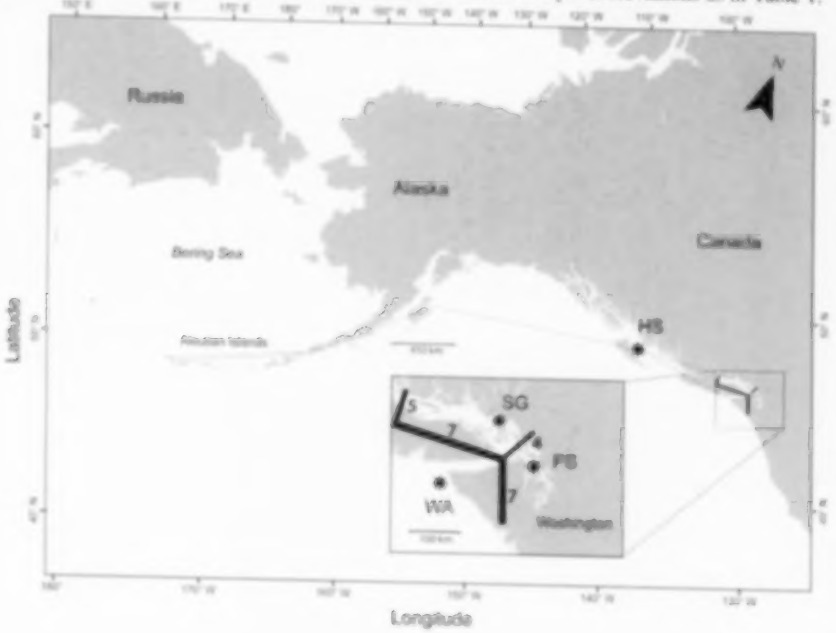
Adult density (no./km)	Mean dispersal distance per generation (km)
1	282.2
10	89.2
100	29.2
1 000	8.9
10 000	0.9
100 000	0.3

the same levels of among-group differentiation (F_{CT}). Results for the three sample locations within Alaska provided some support for differentiation of the Aleutian Islands from the Bering Sea and the Gulf of Alaska: of all possible groupings, the only significant differentiation among groups (F_{CT}) occurred when pooled samples from the Aleutian Islands (AD, AI, and AT) were tested with samples from Unimak Pass (UP03 and UP05) and Kodiak Island (KI03 and KI05; Table 2). AMOVAs conducted on three groupings of temporal replicate samples from the Aleutian Islands (AD, AI, and AT) had F_{CT} values ranging from –0.0010 to 0.0013 that were not significant (data not shown). Likewise, grouping temporal replicates and testing pairs of geographic locations in Alaska produced no significant F_{SC} values.

There were no significant differences in allelic or genotypic frequencies between temporal samples collected at the same sites in Alaska, including the three locations in the central Aleutian Islands archipelago ranging from 75 to 275 km apart (Fig. 1). The joint null hypotheses of no significant genic or genotypic differentiation between sample pairs over all loci were rejected for most comparisons (Table 3), both before (37 of 55 tests) and after (28 of 55 tests) Bonferroni correction. Results of genotypic tests were almost identical (Table 3).

Estimates of F_{ST} and R_{ST} (± standard error, SE) over all samples were 0.005 (±0.002) and 0.010 (±0.003), respectively. No significant differentiation was found among temporal replicate samples or in pairwise geographic comparisons within Alaska (Table 4). Pooling samples from the Aleutian Islands (AD, AI, and AT), Kodiak Island (KI05 and KI03), and Unimak Pass (UP03 and UP05) resulted in significant estimates of differentiation (after sequential correction) between the first two regions and following sequential correction between the latter two regions (data not shown). Pairwise F_{ST} values were generally significant between locations in Alaska and southern coastal locations (Hecate Strait (HS), coastal Washington State (WA), and the Strait of Georgia (SG); Table 4). Pairwise estimates of F_{ST} between Puget Sound (PS) and other locations were generally not significant following sequential correction, and the small sample size (18) undoubtedly resulted in weak power in these comparisons despite relatively high F_{ST} values.

Fig. 4. Delaunay triangulation (dotted lines) with putative barriers to gene flow (solid lines) identified by BARRIER (Manni et al. 2004). Line thickness is proportional to the given number of loci supporting the barrier. Sample abbreviations as in Table 1.



Eight of 11 loci showed significant regression relationships of F_{ST} with geographic distances (data not shown). A Mantel test of linearized multilocus F_{ST} values with geographic distances was highly significant ($P < 0.0001$) and showed clear IBD patterns over the entire sample range in North America (Fig. 2). Over shorter geographic scales, the IBD relationship for samples from Alaska remained highly significant (albeit with lower r^2), with a slope comparable with that over all North American coastal samples combined (Table 5). Samples within the Georgia Basin (SG and PS) consistently yielded higher pairwise estimates of F_{ST} in comparisons with samples obtained from coastal sites than those between coastal sites (Table 4). When these two samples were removed from the regression, the correlation coefficient improved considerably ($r^2 = 0.83$; Table 5). Decomposed pairwise regression analysis showed that mean regression residuals for these two samples were significantly larger than zero (Fig. 3), and Akaike information criterion (AIC) values indicated a best fit when both Georgia Basin samples were excluded (Table 5). Pairwise R_{ST} values also exhibited a significant IBD pattern (Mantel test: $P < 0.005$), although the overall relationship was weaker than for F_{ST} ($r^2 = 0.31$), and again the Strait of Georgia (SG) and Puget Sound (PS) samples appeared to be outliers to the general trend for coastal populations. Estimates of R_{ST} were significantly higher than F_{ST} at three individual loci (*GMA101*, *GMA102*, and *GMA104*) and over all loci ($P = 0.0152$) when the Georgia Basin (PS and SG) samples were included, but not among coastal samples.

Mean dispersal distances estimated from the slope of the IBD regression of F_{ST} values across coastal North America (1.57×10^{-6}) ranged from 0 to 282 km·generation^{-1} over five orders of magnitude in adult densities (Table 6). Excluding an extreme value for a density of 1 individual·linear km^{-1}, mean dispersal estimates for cod were less than 100 km·generation^{-1}.

The BARRIER analysis identified one primary genetic discontinuity supported by seven of 11 loci between the Strait of Georgia (SG) and Puget Sound (PS) from coastal populations (Fig. 4). Other barriers between Puget Sound (PS) and the Strait of Georgia (SG) and the latter location and Hecate Strait (HS) involved four and five loci, respectively. Results from SAMOVA showed that the largest F_{CT} value (0.0269, $P = 0.0215$) was produced by grouping samples from the Georgia Basin (PS and SG) compared with all coastal samples combined, supporting results obtained from BARRIER. Partitioning the samples into three or more groups produced smaller, although significant, F_{CT} values but also introduced a significant within-group variation component.

Discussion

Our microsatellite data revealed one of the clearest IBD patterns reported to date for a marine fish species ($r^2 = 0.83$), demonstrating a monotonic increase of genetic distance with geographic distances along nearly the entire North American range of Pacific cod. Two outlier populations were identified, which appeared to be isolated from the coastal stock complex. Although genetic differentiation was low overall ($F_{ST} = 0.005$), it appeared to be temporally stable. Such patterns suggest limited dispersal in Pacific cod, although pertinent features of species biology and the marine environment need to be considered in the interpretation of our results.

IBD in North American coastal populations

Genetic differentiation among coastal sites indicated the presence of a large stock complex along continental shelves and slopes, with gene flow sufficiently restricted to develop a significant IBD pattern. Tests of genic and genotypic heterogeneity, as well as estimates of F_{ST} and R_{ST}, consistently inferred significant genetic differentiation among populations at distances exceeding ~1700 km along this coastal continuum, a spatial scale comparable with detectable IBD in Atlantic cod (1600 km) in the western North Atlantic (Pogson et al. 2001). Samples from the coasts of Washington State and British Columbia were distinct from those in Alaska and, to a lesser degree, from each other. The IBD pattern among coastal locations across North America is one of the strongest relationships for a marine fish reported to date. Only brown rockfish, *Sebastes auriculatus*, along the California coast showed genetic differentiation that was more closely explained by geographic distance (r^2 = 0.90; Buonaccorsi et al. 2005), while other studies revealed more scatter around a general IBD relationship (e.g., Rocha-Olivares and Vetter 1999).

Patterns of genetic variation reflect both contemporary dispersal and population history (Hutchison and Templeton 1999). A critical assumption of many population genetic inferences is that populations are at migration–drift equilibrium (Slatkin 1993). This assumption may be violated in Pacific cod because of the relatively recent time since colonization of the northeastern Pacific Ocean and Bering Sea following glacial retreat in the late Pleistocene (i.e., <17 000 years for northern populations) and even more recently (ca. 6000–7000 years) for populations in the Georgia Basin (Tunnicliffe et al. 2001). However, IBD patterns may be achieved relatively soon after population separation, depending on the mode of separation (Slatkin 1993). Therefore, the slope of the IBD is often better correlated with direct estimates of dispersal than F_{ST} values themselves because overall levels of genetic differentiation take much longer to reach equilibrium (Rousset 1997). In addition, one-dimensional habitats produce higher levels of divergence and reach migration–drift equilibrium faster than two-dimensional habitats with gene flow in all directions (Crow and Aoki 1984; Pogson et al. 2001). Spawning aggregations of Pacific cod can be found primarily along the 200 m isobath (Dunn and Matarese 1987), and their distribution is thus essentially one-dimensional along continental margins.

Migration–drift equilibrium is also likely to be achieved sooner at shorter distances, resulting in a significant IBD pattern at short, but not at long, geographic distances in non-equilibrium populations (Slatkin 1993). In contrast, the IBD relationship in Pacific cod is constant over different geographic distances, as evidenced by the high r^2 and similarity of slopes along the entire coast and within just Alaska, suggesting that the IBD patterns may be close to equilibrium and thus reflect primarily contemporary dispersal rather than long-term demographic history. Alternative explanations for this IBD relationship, secondary contact among coastal subpopulations or a stepwise recolonization history following glaciation, were not evident in the data.

R_{ST} values were not higher than F_{ST} in North American coastal samples, as would be expected if subpopulations had been isolated, nor was there any geographic trend towards reduced genetic diversity at higher latitudes resulting from postglacial northward expansion of Pacific cod.

Interestingly, broad-scale circulation features of the northeastern Pacific Ocean did not appear to influence geographic patterns of genetic variation. The coastal sample from Washington State (WA) is south of the region where the North Pacific Current bifurcates, forming the northward-flowing Alaska Current and southward-flowing California Current, defining the approximate boundary of the Oregonian and Aleutian zoogeographic provinces (Cummins and Freeland 2007). This current system may be responsible for maintaining a phylogeographic discontinuity between northern and southern populations of rosethorn rockfish, *Sebastes helvomaculatus* (Rocha-Olivares and Vetter 1999). We found no evidence for such a discontinuity in our spatial analyses, suggesting that limited dispersal among subpopulations subject to genetic drift was the primary cause of the observed IBD pattern.

Estimates of dispersal depend on assumptions of average effective densities (Rousset 1997), which can be deducted from recent stock biomass surveys by National Marine Fisheries Service (NMFS). The harmonic mean of stock abundances estimated from 10 trawl surveys between 1984 and 2007 in the Gulf of Alaska was 199 million individuals (Thompson et al. 2007*b*). The coastline of the Gulf of Alaska encompasses approximately 2525 km, yielding an average estimate of 78 800 individuals·km^{-1} coastline. The mean effective density for this area, assuming a conservative ratio of effective to census population size of 0.001 (Hauser et al. 2002; Turner et al. 2002), is therefore roughly 79 individuals·km^{-1}. Realistic dispersal distances may thus not exceed 30 individuals·km^{-1}, comparable with estimates for shallow coastal rockfish species with pelagic larvae (Buonaccorsi et al. 2004, 2005; Miller and Shanks 2004).

Such very short dispersal distances infer very little larval and adult dispersal. Despite the potential for extensive adult movement, Pacific cod may not undertake directed migrations over their lifetime. Cod tagged in the eastern Bering Sea exhibited high site fidelity, with 70% of recaptures occurring within 80 km (Shi et al. 2007). Studies in the Gulf of Alaska have shown that although some fish traveled in excess of 600 km, about 75% stayed within 25 km over considerable time periods (D. Urban, NMFS, Alaska Fisheries Science Center, Kodiak, Alaska 99615, USA, personal communication, 2006). Fish captured during summer months and recaptured during the next spawning period had some of the longest recovery distances, and some tagged and recaptured over two successive spawning seasons had the shortest, suggesting that cod may make extensive annual feeding migrations prior to returning to prior spawning locations or that some stock components are nonmigratory. Archival tagging of Atlantic cod (Svedäng et al. 2007*a*) found evidence for both resident and annually migrating stock components, although whether this represents natal homing is unresolved (Bradbury and Laurel 2007; Svedäng et al. 2007*b*). Larval dispersal may also be limited: Pacific cod lay a single batch of demersal eggs (Hattori et al. 1992) in contrast to the batch-spawning Atlantic cod, which produces planktonic eggs. Larvae remain near the bottom of the water column (Dunn and Matarese 1987), thus potentially aiding larval retention.

Genetic structure in Alaska

The genetic structure of Pacific cod in Alaska is of particular interest, not only because of potential discrepancies between genetic stock structure and established fishery management units, but also because of more complex bathymetric and hydrographic features in the Gulf of Alaska and the Aleutian archipelago than the relatively homogeneous eastern Bering Sea. The IBD relationship observed for three sampled locations in Alaska was temporally stable and similar to the general coastal pattern across the northeastern Pacific Ocean. However, AMOVA results supported the grouping of samples along the contiguous Alaska peninsula (UP and KI) as distinct from those in the central AI region, a relationship that would not be predicted considering only IBD (distance AI–UP, 700–900 km; UP–KI, 1000–1050 km). This pattern of differentiation is generally concordant with meristic stock differences between the eastern Aleutian Islands – Bering Sea and the western Aleutian Islands (Wilimovsky et al. 1967).

Potential factors controlling population connectivity of Pacific cod in the Aleutian archipelago with the much larger stock biomass in the eastern Bering Sea could arise from limits to egg and larval dispersal, as well as oceanographic barriers to movements by adults. Oceanographic circulation in the Aleutian Islands is more complex than in the eastern Bering Sea, resulting in an ecological subdivision at Samalga Pass (Hunt and Stabeno 2005) characterized by differences in ecosystem productivity and species composition and abundance (Logerwell et al. 2005; Stabeno et al. 2005). Fish species with partial distributions across the Aleutian Archipelago often show distinct breaks at zoogeographic provinces separated by deep Aleutian passes (Logerwell et al. 2005), thus inferring potential barriers to population connectivity as well. Vigorous tidal transport through many of these passes creates complex circulation patterns around islands (Stabeno et al. 2005) that could result in entrainment of Pacific cod eggs and larvae at more localized scales than in more homogeneous habitats of the eastern Bering Sea and Northeast Pacific.

Water depth may be an important factor influencing movements by adult (and presumably juvenile) Pacific cod, which are rarely captured by trawl surveys deeper than 300 m (Allen and Smith 1988). Pacific cod are much more demersal in their vertical distributions than Atlantic cod and are typically found less than 10 m from the bottom (Nichol et al. 2007). These two observations suggest that passes in the Aleutian Islands, especially those exceeding 500–1000 m westward of Samalga Pass, may represent significant barriers to dispersal. Deep ocean trenches have been proposed as barriers to migration in other gadid species, contributing to genetic differentiation of Atlantic cod (Bentzen et al. 1996; Ruzzante et al. 1998; Beacham et al. 2002) and haddock (*Melanogrammus aeglefinus*) in the western Atlantic (Zwanenburg et al. 1992). The extent of adult exchange between the Bering Sea and Aleutian Islands regions has not been accurately quantified and may be influenced by suitable depth "corridors" providing habitat connectivity.

Genetic structure in Puget Sound

Pacific cod from the inland waters of the Georgia Basin were markedly differentiated from open coastal locations. Pairwise sample comparisons and spatial analyses inferred strong isolation between populations in fjord environments of the Strait of Georgia (SG) and Puget Sound (PS), a pattern consistent with previous estimates of stock structure derived from tagging studies, acquired markers, and known historical spawning locations (Westrheim 1982; Palsson 1990; Palsson et al.1997). The transboundary Strait of Georgia stock is one of four (including Hecate Strait, Queen Charlotte Sound, and the west coast of Vancouver Island) defined for management purposes in British Columbia (Fisheries and Oceans Canada 1999). Tagging studies (reviewed by Westrheim 1982) showed little movement of tagged cod among three of these regions. Three stocks have been proposed for Puget Sound based on tagging data and spawning locations (Palsson 1990). Differences in parasite frequencies also distinguished Georgia Basin populations from coastal Pacific cod (Westrheim 1987). Gao et al. (2005) reported significant differences in stable isotope ratios of cod otoliths between two sample areas near our coastal Washington State and Strait of Georgia locations.

The existence of fjord populations of Pacific cod is concordant with patterns reported in other marine fishes broadly distributed in the Northeast Pacific, including brown rockfish (Buonaccorsi et al. 2005), copper rockfish (*Sebastes caurinus*; Buonaccorsi et al. 2002), lingcod (*Ophidon elongatus*; Marko et al. 2007), Pacific hake (*Merluccius merluccius*; Iwamoto et al. 2004), and northern clingfish (*Gobbiesox maeandricus*; Hickerson and Ross 2001). Divergence from coastal populations likely arose from, and is maintained by, complex physiographic and circulatory features that restrict egg and larval dispersal. Exchange of deep water with continental shelf waters is restricted by several narrows and sills (Ebbesmeyer et al. 1984; Masson 2002). Similarly, the presence of shallow sills in Norwegian fjords appears to enhance local retention of Atlantic cod eggs (Knutsen et al. 2007), contributing to the development of localized populations (Olsen et al. 2004; Jorde et al. 2007).

The general decline of Pacific cod populations near the southern edge of its historical range in the northeastern Pacific Ocean has prompted concerns regarding their management and conservation (see review in Gustafson et al. 2000). Large declines led to restrictions on both commercial and recreational fisheries after 1987, and the stock has not shown evidence of rebuilding despite extremely low fishing levels since the 1990s. Possible reasons include a warming oceanographic trend during and following the decline (Palsson 1990; Dorn 1993), declines in prey species, and degradation of nearshore subtidal nursery areas (West 1997), or some combination of these factors (see review in Gustafson et al. 2000).

Pacific cod was one of three gadoid species in Puget Sound petitioned for listing under the US Endangered Species Act (ESA) (Wright 1999). In their status review, Gustafson et al. (2000) were unable to identify cod within Puget Sound as a distinct population segment (DPS) under the ESA definition, resulting in a finding of "not warranted" for listing, in part due to insufficient information regarding the northern boundary of a DPS that included Puget Sound (Gustafson et al. 2000). Our study clearly indicates genetic

differentiation of cod from Puget Sound and the Strait of Georgia from coastal populations, although a more detailed delineation and appropriate definition of a potential Puget Sound cod DPS requires further research with larger samples.

In summary, our results refute the paradigm of broad-scale genetic panmixia for Pacific cod in the North Pacific and Bering Sea (Grant et al. 1987) as a consequence of high migration rates (Shimada and Kimura 1994). Instead, our data suggest very limited average dispersal per generation, leading to significant genetic divergence over modest geographic scales (1500–2000 km) along contiguous coastal margins and at lesser distances (hundreds of kilometres) where circulation features and bottom topographies are more heterogeneous. Combined with tagging data, our results potentially may be used to develop a model of cod dispersal to determine the appropriate geographic scales for management and conservation. However, observed departures from IBD in localized inshore stocks suggest caution in this approach. Other isolated fjord populations may exist outside of the Georgia Basin (e.g., Prince William Sound), and more comprehensive sampling of inshore fjords and embayments in the Gulf of Alaska region and throughout the larger Bering Sea – Aleutian Islands management area is needed to more fully resolve the magnitude and geographic patterns of structure in Pacific cod.

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Appendix A

Table A1 follows.

Table A1. Sample size (N), number of alleles (N_A), allelic richness (N_S) corrected to the smallest sample size ($n = 18$), expected (H_e) and observed (H_o) heterozygosities, and estimates of F_{IS} for 11 microsatellite loci in Pacific cod (*Gadus macrocephalus*).

		Sample											
Locus		AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS	ALL
Gma100	N	45	92	45	87	87	106	94	85	69	94	14	818
	N_A	45	68	44	56	60	62	58	56	50	52	14	101
	N_S	18.05	18.12	18.02	17.66	18.16	17.92	17.77	12.26	17.10	16.71	12.54	17.78
	H_e	0.9694	0.9748	0.9694	0.972	0.9753	0.9745	0.973	0.9685	0.9659	0.9642	0.9056	0.978
	H_o	0.9778	0.9674	0.9333	0.908	0.9655	0.9717	0.9787	0.9294	0.9565	0.9255	0.8571	0.950
	F_{IS}	0.003	0.013	0.048	0.072	0.016	0.008	–0.001	0.046	0.017	0.045	0.090	0.029
Gma101	N	45	92	45	87	94	106	92	84	69	94	11	819
	N_A	16	21	16	23	23	25	23	22	19	21	12	38
	N_S	10.81	11.17	10.89	11.52	11.33	11.85	11.98	11.24	10.66	10.59	12.00	11.43
	H_e	0.9099	0.9143	0.9086	0.918	0.9179	0.9188	0.9208	0.9138	0.9041	0.9035	0.8678	0.920
	H_o	0.8667	0.8913	0.9556	0.9425	0.9255	0.8868	0.9022	0.9405	0.8696	0.8723	0.9091	0.905
	F_{IS}	0.059	0.031	–0.040	–0.021	–0.003	0.040	0.026	–0.023	0.046	0.040	0.000	0.017
Gma102	N	45	92	45	87	95	106	93	89	69	94	16	831
	N_A	14	15	13	16	13	17	14	14	13	14	11	19
	N_S	9.28	9.62	9.68	9.62	9.03	9.89	9.51	9.43	9.04	9.08	9.82	9.47
	H_e	0.8686	0.8898	0.8825	0.8859	0.8811	0.8991	0.8922	0.8887	0.8766	0.8793	0.8516	0.892
	H_o	0.9111	0.9239	0.9333	0.9655	0.8737	0.9245	0.9032	0.8539	0.8841	0.8511	0.75	0.898
	F_{IS}	–0.038	–0.033	–0.046	–0.084	0.014	–0.024	–0.007	0.045	–0.001	0.037	0.151	–0.006
Gma103	N	43	92	44	87	94	106	94	88	69	94	17	828
	N_A	30	37	32	36	38	38	39	39	37	41	20	59
	N_S	13.15	13.38	14.26	11.78	12.70	12.09	11.85	13.53	13.76	14.10	15.02	13.18
	H_e	0.9143	0.9301	0.9243	0.8986	0.9091	0.8991	0.8917	0.9245	0.9239	0.9374	0.9143	0.923
	H_o	0.9302	0.8913	0.9318	0.908	0.9574	0.9245	0.8617	0.9545	0.9275	0.9362	0.9302	0.920
	F_{IS}	–0.006	0.047	0.003	–0.005	–0.048	–0.024	0.039	–0.027	0.003	0.007	0.070	0.003
Gma106	N	45	90	45	87	95	106	94	89	69	94	16	830
	N_A	8	12	10	12	13	10	13	10	11	12	8	17
	N_S	5.70	6.43	6.52	6.89	7.81	7.38	7.37	6.57	6.63	6.73	6.66	6.94
	H_e	0.7353	0.7404	0.7968	0.8063	0.8333	0.8257	0.8153	0.8034	0.8024	0.8061	0.7539	0.812
	H_o	0.7111	0.7556	0.8667	0.7931	0.8316	0.8585	0.8191	0.8315	0.7246	0.8191	0.875	0.807
	F_{IS}	0.044	–0.015*	–0.077	0.022	0.007	–0.035	0.001	–0.029	0.104	–0.011	–0.129	0.006
Gma108	N	45	92	45	87	94	106	94	89	69	92	17	830
	N_A	8	11	10	10	14	12	11	11	9	9	5	20
	N_S	4.25	4.59	5.11	4.56	5.16	5.15	4.74	5.15	3.59	4.66	3.82	4.75
	H_e	0.4247	0.4637	0.5504	0.4345	0.5171	0.4717	0.4388	0.5463	0.2871	0.5178	0.2664	0.469
	H_o	0.4000	0.5000	0.5778	0.4138	0.5532	0.4151	0.4894	0.5618	0.2464	0.5109	0.2353	0.465
	F_{IS}	0.069	–0.073	–0.039	0.054	–0.064	0.125	–0.11	–0.023	0.149	0.019	0.147	0.009
Gma109	N	44	91	44	87	94	106	94	85	69	94	11	819
	N_A	21	29	22	29	26	28	26	26	24	24	14	35
	N_S	11.58	11.72	12.29	12.71	11.99	12.13	11.55	12.55	12.63	12.13	14.00	12.37
	H_e	0.9021	0.9108	0.9179	0.9278	0.9215	0.9235	0.9082	0.9285	0.9288	0.9243	0.8802	0.929

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Table A1 (*concluded*).

Locus		Sample											
		AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS	ALL
	H_o	0.9091	0.9011	0.8864	0.9195	0.9149	0.8774	0.8723	0.9176	0.8841	0.9362	0.8182	0.901
	F_{is}	0.004	0.016	0.046	0.015	0.012	0.055	0.045	0.018	0.055	–0.008	0.118	0.030
Gmo37	N	45	92	45	87	95	106	93	89	69	94	16	831
	N_A	26	36	25	32	36	36	34	31	27	32	12	65
	N_S	13.33	13.63	12.97	12.88	13.68	13.15	13.27	12.26	11.84	12.59	9.76	12.98
	H_e	0.9289	0.933	0.919	0.9242	0.9368	0.9247	0.9307	0.9051	0.9038	0.9165	0.8184	0.928
	H_o	0.9556	0.8913	0.9333	0.9310	0.9579	0.8962	0.9140	0.9101	0.9565	0.9468	0.7500	0.923
	F_{is}	–0.017	0.050	–0.034	–0.002	–0.017	0.036	0.023	0.000	–0.051	–0.028	0.115	0.006
Trh20	N	45	92	45	87	94	106	93	89	69	93	16	829
	N_A	20	21	23	22	22	24	22	21	22	21	13	27
	N_S	12.24	12.35	13.87	12.62	12.16	12.63	12.58	11.60	12.73	11.89	11.37	12.38
	H_e	0.9222	0.9311	0.9407	0.9322	0.9284	0.9343	0.9343	0.9213	0.9329	0.9262	0.8984	0.936
	H_o	1.0000	0.9130	0.9778	0.9540	0.9362	0.9057	0.9462	0.9326	0.9710	0.9892	0.9375	0.947
	F_{is}	–0.073	0.025	–0.028	–0.018	–0.003	0.035	–0.007	–0.007	–0.034	–0.063	–0.011	–0.012
All loci	N	44.67	91.67	44.78	87.00	93.56	106.00	93.44	87.44	69.00	93.67	14.89	
	N_A	220	282	225	268	285	283	276	263	242	255	125	
	N_S	10.74	10.83	11.10	10.78	11.06	10.89	10.86	10.65	10.55	10.32	9.97	
	H_e	0.8576	0.8624	0.8772	0.8651	0.8764	0.8684	0.8651	0.8709	0.8482	0.8610	0.8292	
	H_o	0.8521	0.8490	0.8926	0.8566	0.8790	0.8542	0.8593	0.8734	0.8340	0.8490	0.7874	
	F_{is}	0.006	0.015	–0.018	0.010	–0.003	0.016	0.007	–0.003	0.017	0.014	0.005	

Note: Sample abbreviations as in Table 1. *, $P \leq 0.05$.

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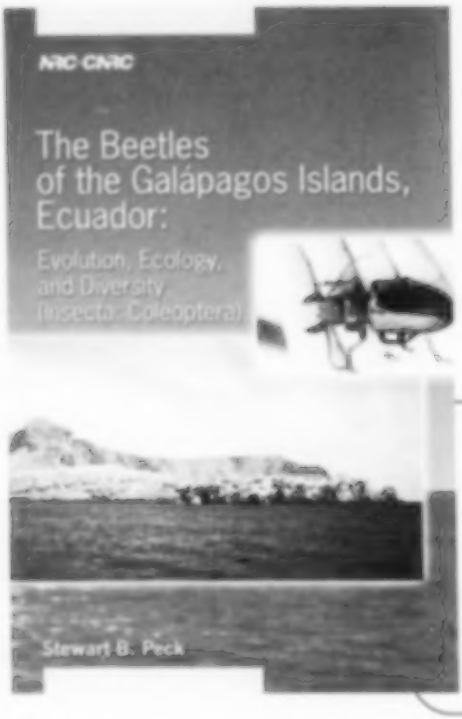
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Evolution, Ecology, and Diversity (Insecta: Coleoptera)
Stewart B. Peck

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By / Par
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ANNOUNCEMENT

The Timothy R. Parsons Award for Excellence in Ocean Sciences

Fisheries and Oceans Canada (DFO) has established the Timothy R. Parsons Award for excellence in Ocean Sciences to recognize a Canadian scientist for outstanding lifetime contributions to multidisciplinary facets of ocean sciences or for a recent exceptional achievement, while working within a Canadian institution. This award is named after Dr. Timothy R. Parsons and honours his distinguished career as a Fisheries Research Board of Canada researcher, university professor, broadly read author, and recipient of the 2001 Japan Prize. For more information on Dr. Timothy R. Parsons, please visit **http://www.dfo-mpo.gc.ca/science/award-prix/parsons/index-eng.htm**

Eligibility Criteria

The Timothy R. Parsons Medal is

- awarded to a Canadian for distinguished accomplishments in multidisciplinary facets of ocean sciences;
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No posthumous nominations are considered.

To make a nomination, please complete the appropriate nomination form on the Parsons Award website at
http://www.dfo-mpo.gc.ca/science/award-prix/parsons/about-apropos-eng.htm

Nominations should be received no later than **February 28** of every year at

Timothy R. Parsons Medal Committee
Strategic Science Outreach
Stn. 8W135 – 200 Kent Street
Ottawa, Ontario, K1A 0E6
Tel: (613) 991-1021
FAX: (613) 990-5113
Email: sciencebulletin@dfo-mpo.gc.ca

COMMUNIQUÉ

Mise en candidature pour le prix Timothy R. Parsons
Pour l'excellence en sciences de la mer

Pêches et Océans Canada a créé un prix visant à rendre hommage à l'excellence dans le domaine des sciences de la mer au Canada. Ce prix porte le nom de Timothy R. Parsons, en l'honneur de la carrière remarquable de ce chercheur du Conseil de recherches sur les pêcheries du Canada, professeur d'université, auteur de publications prestigieuses et lauréat du Prix du Japon en 2001. La médaille Timothy R. Parsons est décernées à des scientifiques canadiens qui se sont distingués dans un domaine pluridisciplinaire lié aux sciences de la mer et souligne l'ensemble de leur carrière ou une réalisation récente exceptionnelle au sein d'un établissement canadien. Pour plus d'information au sujet de Timothy R. Parsons, visitez le site web au **http://www.dfo-mpo.gc.ca/science/award-prix/parsons/index-fra.htm**

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